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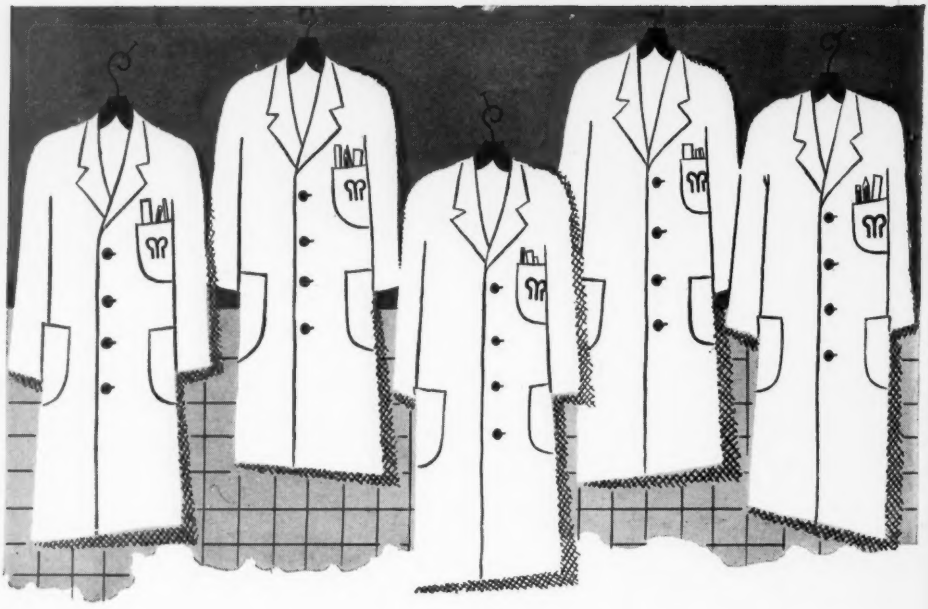

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THE USE OF POTASSIUM IN THERAPY*

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THE title of this discussion should perhaps be broadened to "the background for the use of potassium as a therapeutic agent, and some clinical experiences with potassium administration." In the case of a pharmacologic agent, foreign to the organism, one might begin such a topic with the functional alterations resulting from administration of the substance. But in the case of potassium, one is dealing with an element which is present in large quantity in the animal organism, and which constitutes the major basic element of chemical structure of the cell. Thus a review of current knowledge of the metabolism of this substance, both normal and abnormal, is essential for an intelligent approach to its use as a therapeutic agent. It warrants emphasis that, though the present discussion will highlight the activities of this single structural element, other components such as protein, phosphorus, sulfur and magnesium, deserve equal consideration in the preservation of the functional efficiency of the cell.

The cell is the basic structural unit of the human organism. Within its confines enzymatic and other metabolic processes furnish the energy for

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heat, growth, muscular work and the multiple special functions in the highly organized systems. Potassium is the chief basic ion (or cation) of the cells, its concentration being variously estimated between 140 and 160 mEq. per liter of cell water (1). Outside the cells, in the vascular and perivascular spaces, sodium is the chief cation, and normally potassium exists here in a concentration of only 4.0 to 5.5 mEq. per liter. Between the two compartments, there must be maintained ionic, osmolar and acid-base equilibria. Under certain abnormal circumstances, as will appear later, some sodium can invade the cells and replace potassium there; but available evidence indicates that there is a reduction of the functional efficiency of the cells when this has occurred.

It has recently been demonstrated by Folk, Zierler, and Lilienthal (2) that the potassium concentration of the extracellular fluids is accurately reflected by the plasma concentration of this element, according to the Gibbs-Donnan equilibrium. This is an exceedingly fundamental piece of information, for unless the readily available plasma could be used for potassium determination, one would have no way of knowing the concentration of potassium in the fluid actually bathing the cells.

The concentration of potassium in the extracellular compartment is clearly the resultant of how much of this element comes in and how much goes out. Potassium can enter this compartment by way of the gut—absorption of ingested potassium—or it can emerge from the cells. Potassium can leave the extracellular space by entering the cells or by excretion in the urine. Only in diarrheal states are significant amounts of potassium lost by way of the stools. Gastric juice (and perhaps juices of the small intestine as well) contains potassium in concentration several-fold greater than the plasma. We have found this true in patients with and without free hydrochloric acid, and it has only been in patients suffering from very low serum potassium levels that gastric juice has contained less than 10 mEq. per liter; usually from 15 to 25 mEq. (3). Thus excessive vomiting or prolonged gastric suction might prove to be ways of losing considerable quantities of potassium. Homeostatic mechanisms are so delicately arranged that it requires a major functional disturbance to alter the concentration of potassium in the extracellular fluids from its normal range. When elevation or depression of the potassium concentration does occur, however, serious consequences ensue.

It has long been known that alterations in the potassium concentration in the fluids bathing the heart cause disturbances in cardiac mechanisms. Ringer (4), in developing his solution with which to perfuse the isolated mammalian heart, found that either too high or too low a concentration of potassium interfered with the normal function. Development of electrocardiography, together with accurate methods for the measurement of

potassium on small samples of biologic materials, brought about recognition of the changes in cardiac mechanisms coincident with high and low concentrations of potassium in the serum (5–16). The almost universal availability of the electrocardiogram renders the cardiographic record the most practical method to the clinician for the detection of hyper- and hypokalemia. For the benefit of those not familiar with the diagnostic points involved, a brief schematic series of sketches is shown (Fig. 1).[1]

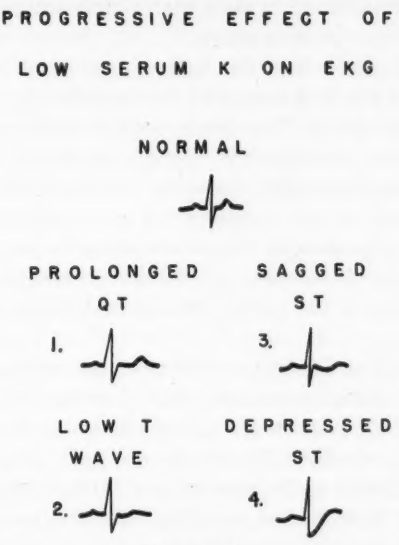

FIGURE 1

Effects of potassium on the human electrocardiogram: The changes in the electrocardiogram produced by abnormalities of the serum potassium are characteristic and easily recognized. As the serum potassium is lowered, prolongation of the Q-T interval is the first change seen in the electrocardiogram. The Q-T interval varies with the heart rate, but at average heart rates it should be less than one-half the R-R interval and this can be used as a rough upper limit of normal. It is well known that hypocalcemia prolongs the Q-T interval, but Nadler (14) and others have shown that the low potassium effect is independent of serum calcium. Nadler suggests that the Q-T interval prolonged by low potassium can be distinguished from that prolonged by low calcium. The low broad T wave of hypokalemia is primarily responsible for the prolongation of the Q-T interval, while in hypocalcemia the T wave is normal and the lengthened

[1] For these sketches and the description in the succeeding paragraphs, we are indebted to Dr. R. S. Ross.

ST segment produces the change. The other changes in the ST segments seen with low potassium are also of differential value. Digitalis and myocardial disease influence the Q-T interval and these factors should be considered in interpreting this measurement.

The second change is lowering or inversion of the T waves and in the third stage the ST segments sag. In the final stage the take-off of the ST segments is depressed and there is a slow staircase-like rise to a low late T wave. This final stage is seen with potassium levels in the range of 1.5 milliequivalents per liter or lower.

With high serum potassium the first change seen in the electrocardiogram is elevation of the T waves, and this is followed by a decrease in the amplitude of the R waves. The third stage is characterized by auricular arrest and no P waves are seen. At slightly higher levels the ST segments become depressed and the QRS duration increases. In the terminal stage, with potassium levels in the range of 9.5 to 10 milliequivalents per liter, the QRS becomes so prolonged that it blends with the elevated T wave to give a biphasic curve. These broad "sine wave" complexes may occur with complete irregularity as the auricular pacemaker has dropped out.

Peripheral, as well as cardiac, neuromuscular mechanisms are disturbed as the result of abnormal concentrations of potassium. Curiously enough, the neurologic manifestations are much the same whether the potassium be high or low. Sensory disturbances are minimal, usually with complaints of only mild paresthesias of the extremities. Motor phenomena are striking; first weakness and then flaccid paralysis of the extremities, sometimes of the ascending or Landry's type. The trunk and cranial nerves are rarely involved but weakness of the respiratory muscles results in a shallow, regular, rapid pattern which is distinctive. However, these peripheral neuromuscular defects are late manifestations of hyper- or hypokalemia and are always preceded by electrocardiographic changes. Conversely, in recovery from the dysfunction of hypokalemia, the electrocardiogram shows reversion to normal soon after return of the serum potassium to normal, whereas the paralytic phenomena pass far more slowly and, indeed, may persist for several hours.

Experimental work on potassium metabolism in animals has yielded much important information, of which space permits summarization of but a few points. Orent-Keiles and McCollum found that, on diets very low in potassium, rats died within three weeks (17). Postmortem analysis revealed the potassium content of heart, skeletal muscle and kidney to be greatly reduced, as much as one-third; amazingly enough, the liver potassium was normal. In these animals Follis found necrosis and scarring of the heart

muscle fibers, as well as necrosis of renal epithelium with tubular dilatation (18). Low serum potassium, low muscle potassium and myocardial necroses have been produced also by poisoning with desoxycorticosterone, presumably in large part as the result of the potassium diuresis effected by this steroid (19, 20, 21).

Darrow and his co-workers (22, 23, 24), using a variety of techniques to induce lowering of the cellular potassium, confirmed previous evidence that there exists a reciprocal relationship between sodium and potassium in muscles; that is, whenever muscle potassium was lowered, there was a corresponding increase in the sodium content. They found in their rats also that whenever a decrease in muscle potassium of more than 10 per cent was present, there was invariably a significant lowering of the concentration of potassium in the serum. Of considerable interest was their observation that potassium administered to potassium depleted animals was retained; whereas, when potassium was injected into normal rats, muscle content was increased only momentarily, and the balance of potassium was soon actually negative. Recently Darrow has pointed out that in their experimental animals there existed a high degree of correlation between the concentration of bicarbonate and chloride in the serum, and the muscle content of sodium and potassium. High serum bicarbonate and low serum chloride invariably accompanied low muscle potassium and high muscle sodium, whether the latter state had been induced by low potassium diet, desoxycorticosterone or the administration of sodium salts (25). It was emphasized by Darrow and co-workers that these predictable relationships were found to apply only after "biological equilibrium" had been reached, a term implying that renal function was being normally carried out. Thus, as in practically every pathologic state, the presence of renal insufficiency greatly complicates interpretation of the chemical anatomy of the body fluids.

To summarize the data presented thus far: There is experimental evidence that 1) the organism is incapable of adequately withholding potassium in the face of a prolonged dietary deficiency of this element; 2) that this potassium deficit is accentuated by coincident administration of sodium salts or by desoxycorticosterone; 3) that reduction of cellular potassium below a certain point is dangerous not only in causing muscular (myocardial) necroses, but that serum potassium is also lowered. Recognition of hypokalemia may be obtained through electrocardiography, and is to be suspected in symmetrical peripheral motor palsies with little or no sensory changes. Potassium depletion should be suspected when, in the presence of normal kidney function, there is an unexplained high serum bicarbonate and low chloride concentration.

CLINICAL OBSERVATIONS

It has been known for more than ten years that some abnormality in potassium metabolism plays an important role in the attacks of familial periodic paralysis (26). The serum potassium is found to be lowered during the paralytic phases, and attacks may be induced by such physiologic "potassium-lowering" procedures as a glucose test meal, administration of insulin or by epinephrine (27, 28, 29). Furthermore, both spontaneous and induced episodes are promptly cured by potassium salts. The exact nature of the underlying physiologic defect in this condition is still unknown. There is no apparent deficiency of cellular potassium.

Hypokalemia, sufficiently severe to produce paralytic phenomena, has been observed to follow overzealous administration of desoxycorticosterone to patients with Addison's disease (30). Recovery followed the oral use of potassium chloride. Here, of course, there was presumptive depletion of cellular potassium, as is found in the experimental animal poisoned with desoxycorticosterone (19, 24). In the normal adult economy, almost the same quantity of potassium is excreted in the urine as has been ingested. In most types of renal insufficiency, so long as urinary volume is maintained, the capacity to excrete potassium remains adequate to cover the requirements; and clinically significant deviations from the normal concentrations of potassium in the serum do not occur unless unusually heavy loads are thrown on the mechanism, such as administration of potassium salts. In oliguric uremia, elevation of serum potassium is not unusual; indeed death from hyperkalemia is probably more common than is generally recognized. Occasionally a type of disturbed renal mechanism is met which results in the urinary output of greater quantities of potassium than normal; there is thus brought about a depletion of potassium with the usual neurologic and electrocardiographic signs thereof (31, 32). Prompt recovery from the immediate presenting symptoms occurs with administration of potassium salts.

In the remaining examples of hypokalemia to be discussed, there are associated profound disorders of nutrition. As a background for an understanding of these cases, it would therefore seem wise to review some of the data on the metabolism of potassium in starvation and recovery therefrom. When no food is eaten, there is expenditure of cellular elements by the body for energy, heat and repair. In Benedict's experiment (33), in which his professional "faster" took nothing but water for thirty days, the amounts of nitrogen, potassium, phosphorus and sulfur that appeared in the urine closely paralleled the relative proportions of these elements in muscle protoplasm. During his month of starvation, Benedict's patient "consumed" and excreted between 15 and 20 per cent of his protoplasmic

mass. Gamble, Ross and Tisdall's studies on children also showed great losses of intracellular elements during fasting (34). Observations made during World War II indicated that the same type of parallel expenditure of cellular elements accompanied brief starvation periods as well as periods of graded undernutrition, provided the diet was kept *qualitatively* constant (35). But during recovery—that is, when these patients were fed full diets —it was found that potassium was retained sooner and in greater quantity than was nitrogen, even though nitrogenous foods were offered liberally. A similar retention pattern was noted when obese patients were starved for four days and then fed 1200 calorie diets (36).

During experiments with total intravenous feeding, it seemed logical to several members of the group studying the metabolic aspects of convalescence (37) to include salts of potassium along with glucose and the nitrogenous pabulum of protein hydrolysates, thus at least offering to the cells another of their normal constituents. It was found that potassium was accepted under these circumstances, and often in considerable quantities. Our group, since 1944, have felt it rational to add from 3 to 8 grams of potassium chloride per day (40 to 110 mEq. of potassium), with corresponding reduction of sodium chloride, to all total intravenous feeding mixtures (except in patients with anuria or renal insufficiency of the potassium-retaining type).

Stewart and Rourke (38) had noted in 1942 that, in postoperative patients fed only parenterally, more potassium was lost in the urine of a patient given normal saline than was lost by a patient to whom only glucose solutions were given. Mason and Howard (39) had noted that sodium and chloride were retained postoperatively in amounts seemingly far greater than were needed for simple repletion of the extracellular space; and, since exaggerated retention of sodium chloride occurred during simple oral feeding after brief starvation periods (36), it seemed likely that avidity for sodium and chloride was a phenomenon of undernutrition. If then, in undernutrition, there is depletion of, along with other things, potassium; and administration of sodium causes great retention of sodium, and potassium diuresis (40), why were we not frequently observing hypokalemia among undernourished patients who were being given only sodium chloride and glucose parenterally? The answer is that we were seeing such cases but not recognizing them; for the significance of much of these data was not realized at the time.

Infantile diarrhea

The administration of potassium salts with the avowed purpose of replenishing cellular supply or preventing further vital depletion was reported from Darrow's laboratory in the treatment of infantile diarrhea.

The exhaustive studies on potassium and sodium interrelationships made by Darrow's group (20, 22, 23, 24, 25), and the knowledge that relatively large quantities of potassium were lost in diarrheal stools, led them to the use of potassium salts parenterally in the treatment of infantile diarrhea. Previously, standard therapy had included only sodium salts. In the first sizeable group of cases treated by the newer method, the mortality was reduced from 32 per cent to 6 per cent (41).

Diabetic acidosis

Since, in the studies on convalescence (37), our attention was being directed toward the provision to the cells of elements that had been depleted and might be accepted and beneficial, in 1945 potassium salts were added to the infusions given to patients with diabetic acidosis. There was, at the time, no conscious effort to forestall the development of hypokalemia. It had been known, since the classical studies of Atchley, Loeb, Benedict, Richards and Driscoll (42) on diabetic volunteers, that during the development of insulin-withdrawal acidosis, diuresis of large quantities of potassium, nitrogen, phosphorus and other elements occurs, much like the pattern of starvation. The study by Atchley's group had also demonstrated that, with resumption of insulin, there was a sharp retention of all the previously lost elements, especially conspicuous in the case of potassium. Their patients had, of course, been fed constant diets during the experimental periods, and insulin was resumed when the metabolic situation seemed precarious. The first few patients with diabetic acidosis studied here, to whose intravenous therapy potassium salts were added, manifested strongly positive potassium balance without elevation of serum potassium, an indication that the majority of the administered potassium ions had been taken up by the cells (43). When it became apparent from the papers of Holler (44), Martin and Wertman (15), Frenkel, Groen and Willebrands (45), Nicholson and Branning (46), and Logsdon and McGavack (47) that, with the current therapeutic regimens, patients recovering from diabetic acidosis might be seriously endangered by the development of hypokalemia, the quantities of potassium given to our patients were increased to see just how much the cells might be capable or "desirous" of accepting. We were quite unprepared for the enormous positive balances shown by some of our cases (43). In one instance, as much as 35 grams of potassium chloride (467 mEq. of potassium) was injected into a patient in eighteen hours; and less than 100 mEq. potassium appeared in the urine. During this time the serum potassium was constantly subnormal. It has been our impression that patients given large doses of potassium and far less sodium than with currently common therapeutic regimens, have profited greatly, not only in the prevention of the dangerously low levels

of potassium which sometimes develop, but the patients have seemed to show a quicker recovery to fitness and regulation. There has also been in the patients so treated a conspicuous absence of edema and hypoproteinemia, which were common aftermaths of our former therapeutic procedures.

Chronically ill, undernourished patients

As previously mentioned, for several years patients under the nutritional study by our group have been given parenteral feeding mixtures which included moieties of potassium. It seemed likely that, among debilitated and depleted persons to whom only saline, glucose and perhaps plasma or amino acids were given, there might be found a fair number who would show evidences of hypokalemia. Last summer, with the aid of surgical colleagues, patients of this type were sought. The following cases are representative of this group:

J.P., J.H.H. #474293, was a suspect because of an unexplained high serum bicarbonate and a low serum chloride, against which efforts at correction by normal saline and ammonium chloride had been unavailing. He was a man aged 53, who had been ill for over a month before entering the Johns Hopkins Hospital. There had been constant abdominal pain which permitted only minimal ingestion of food. Weight loss had been greater than 20 pounds. After hydration with sodium chloride and glucose solutions, abdominal operation was performed on September 20, and there was found pancreatic necrosis, abscess formation, perforated gall bladder and infarction of the omentum. During the postoperative course, practically nothing could be taken by mouth and parenteral feedings consisted of glucose, saline, plasma and whole blood (Fig. 2 and Table 1). As the serum chloride was noted to be falling and the bicarbonate rising, sodium chloride therapy was pushed, and ammonium chloride added—without improvement (October 1–4). On October 5, examination in consultation showed the patient to be extremely asthenic; respirations were shallow but only slightly more rapid than normal; peripheral reflexes were barely obtainable; blood pressure was normal, and there were no evidences of "shock" or heart failure. The cardiogram disclosed definite evidence of hypokalemia.

On October 6, the morning that potassium therapy was begun, the serum potassium was found to be 1.8 mEq. per liter. A mixture of glucose and "Elamine,"[2] to which potassium chloride was added, was given by vein each day over periods of eight to ten hours (Fig. 2 and Table 1). The first day 4 grams of potassium chloride was given. No sodium was included in the mixture except on October 12 and 13, when 500 cc. of normal saline was added.

There was no apparent change in the patient's condition, nor in the chemical pattern of the plasma until October 12, the seventh day of potassium therapy, at which time there began a dramatic improvement in both. He began to feel progressively stronger, appeared more alert and reflexes became readily obtainable. On October 14 the serum potassium had reached a level of 4 mEq. per liter, chloride was rising and CO_2 falling; serum sodium, which had fallen during the intravenous potassium period, was rising. Intravenous feed-

[2] Elamine is a protein hydrolysate which contains minimal quantities of sodium chloride. The material was furnished through the courtesy of the Interchemical Corporation.

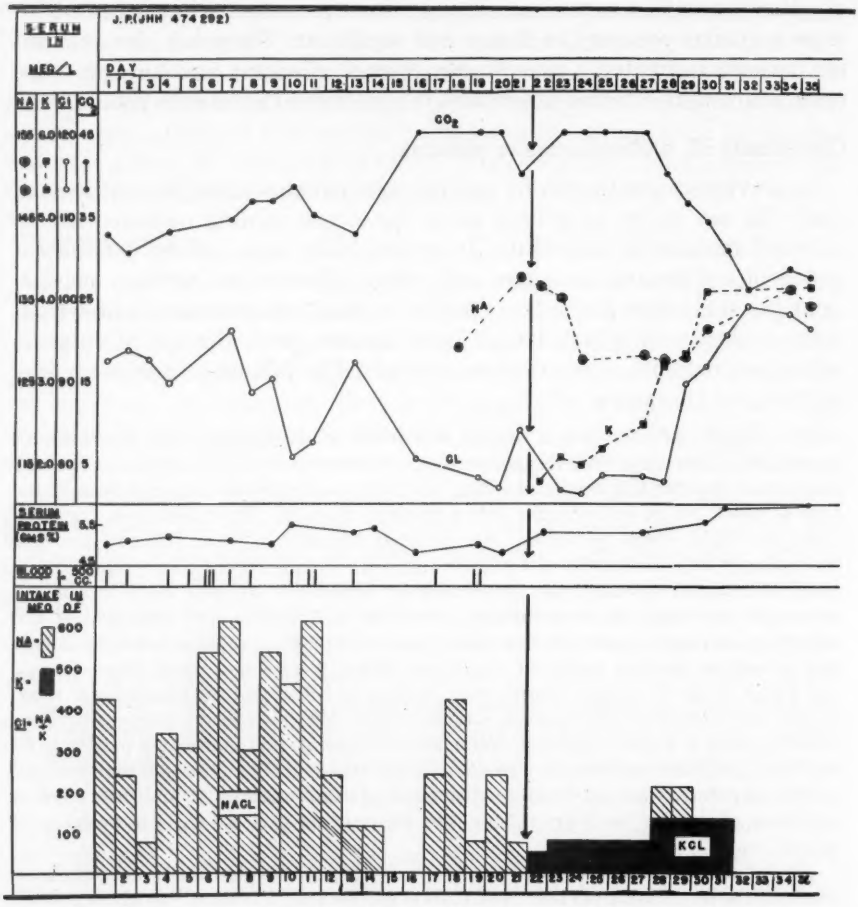

FIG. 2. Electrolyte pattern of serum in case J.P. Intake of sodium prior to administration of potassium was over 6000 mEq. On day 28 and 29, 145 mEq. of sodium was given. Total intake of potassium was 991 mEq.

ing was stopped, and the patient was given 10 grams of potassium chloride orally, together with a routine surgical liquid diet.

It is most unfortunate that urinary incontinence prevented the collection of balance data on this patient. The serum CO_2 determinations prior to October 8 were recorded by the laboratory simply as "greater than 40 mEq." and the actual height reached is unknown. The long latent period (six days) before either clinical or laboratory improvement became evident was a source of discouragement, but subsequent cases have led us to expect this phenomenon. There seemed to be adequate urinary volume throughout this period; no edema was ever apparent, nor was there evidence either of shock or cardiac

TABLE 1

J.P. (J.H.H. #474293)

Date 1948	Serum Values Cl mEq./L	CO_2 mEq./L	Na mEq./L	K mEq./L	P mg./100 cc.	Ca mg./100 cc.	Total protein Gm./100 cc.	NPN mg./100 cc.	Intravenous intake Blood cc.	Na mEq./L	K mEq./L	Remarks
9–15	92.5	32.8					5.0	25	500	422.37		Admitted: sick for six weeks: little or no food, nausea or vomiting
9–16	93.8	30.8					5.1		500	240.85		
9–17	92.6							23		76.95		
9–18	89.9	33.0					5.2 (2.9)	22	500	346.3		
9–19									500	307.8		Levin tube
9–20									1500	538.6		Operation
9–21	96.0	34.7					5.1		250	615.6		
9–22	88.6	36.8						23	750	300.1		
9–23	90.3	36.8					5.0	14		538.6		
9–24	80.7	38.5					5.5	22	150	461.7		
9–25	82.6	35.1						22	1350	615.6		
9–26										230.8		
9–27	92.2	32.8					5.3	30	500	115.4		
9–28						7.8	5.3	20		115.4		
9–29												
9–30	80.8	40.0					4.8	28				
10–1									500	240.85		
10–2			129.0							423.30		
10–3	78.4	40.0					5.0 (2.8)	22	1000	79.90		Bilateral ligation of superficial femoral veins
10–4	77.0	40.0			1.6	8.7	4.8 (2.9)	22		240.85		
10–5	84.0	40.0	137.5							76.90		5985 mEq. Na + Cl to date
10–6			136.3	1.8	1.0		5.3 (3.6)	24			53.6	Potassium therapy begun
10–7	76.8	40.0	135.0	2.1				30			80.4	Miller-Abbott tube started
10–8	76.5	45.0	127.5	2.0							80.4	
10–9	78.5	45.0		2.2							80.4	
10–10											80.4	
10–11	78.3	45.0	128.0	2.5			5.3				80.4	
10–12	77.7	40.0	127.5	3.2						76.95	134.0	
10–13	89.6	36.4	127.7	3.35	0.5					76.95	134.0	773 mEq. K to date
10–14	91.8	34.3	131.2	4.1			5.6	22				Oral administration begun; i.v. stopped
10–15	95.4	30.8			0.7	8.8	5.9 (3.0)				134.0	991 mEq. K to date
10–16	99.0	26.2										
10–17												
10–18	98.2	28.4	136.2	4.95								
10–19	96.2	27.6	133.8	4.15			6.1 (3.3)					

TABLE 2

E.A. (J.H.H.#467811)

Date	Intake							Output							Balance						
	Na mEq./L	K mEq./L	Ca mg./100 cc.	Cl mEq./L	P mg./100 cc.	N Gm./100 cc.	Fluid cc.	Na mEq./L	K mEq./L	Ca mg./100 cc.	Cl mEq./L	P mg./100 cc.	N Gm./100 cc.	Fluid cc.	Na mEq./L	K mEq./L	Ca mg./100 cc.	Cl mEq./L	P mg./100 cc.	N Gm./100 cc.	Fluid cc.
Nov. 3–4	101	112	423	149	915	12.5	3250	99	44	94.5	147	356	9.9	1295	+ 2	+68	+329	+ 2	+559	+2.6	+1995
4–5	101	112	423	149	915	12.5	3250	74	36	182.0	111	312	9.2	1000	+27	+76	+241	+ 38	+603	+3.3	+2250
5–6	101	112	423	149	915	12.5	3250	89	21	154.0	92	75	6.4	860	+12	+91	+269	+ 57	+840	+6.1	+2390
6–7	101	112	423	149	915	12.5	3250	33	38	109.0	49	178	8.4	950	+68	+74	+314	+100	+737	+4.1	+2300
7–8	93	103	389	138	843	11.5	3000	75	44	141.0	62	342	8.5	1050	+18	+59	+248	+ 76	+501	+3.0	+1950
8–9	96	106	403	143	852	11.9	3100	102	74	280.0	100	328	10.4	1640	− 6	+32	+123	+ 43	+524	+1.5	+1460
9–10	101	112	423	143	915	12.5	3250	104	79	232.0	108	462	9.3	1540	− 3	+33	+191	+ 35	+453	+3.2	+1710
10–11	101	112	423	143	915	12.5	3250	119	89	238.0	140	644	10.6	2060	−18	+22	+185	+ 3	+271	+1.9	+1170
11–12	101	125	423	163	915	12.5	3250	79	65	180.0	95	526	7.6	1200	+22	+60	+243	+ 68	+389	+4.9	+2050
12–13	101	138	423	176	915	12.5	3250	105	88	240.0	129	552	9.7	1380	− 4	+50	+183	+ 47	+363	+2.8	+1870
13–14	101	138	423	176	915	12.5	3250	86	114	288.0	140	567	11.4	1620	+15	+24	+135	+ 36	+348	+1.1	+1630
14–15	101	138	423	176	915	12.5	3250	77	102	258.0	134	603	11.6	1340	+24	+36	+165	+ 42	+312	+0.9	+1910
15–16	97	133	406	169	878	12.0	3150	20	102	216.0	118	434	10.1	1160	+77	+31	+190	+ 51	+444	+1.9	+1990

decompensation. Once the chemical pattern began to change, the rapidity of its return to normal was astonishing and most gratifying. Even the serum protein concentration, which had been between 5.0 and 5.3 grams per 100 cc. since September 15 despite transfusion of 7 liters of blood, rose to 6.1 by October 19, coincident with the rises in serum potassium and chloride and the fall in CO_2. Serum phosphorus, very low when potassium

TABLE 3

E.A. (J.H.H. #467811)

Date 1948	Cl mEq./L	CO_2 mEq./L	Na mEq./L	K mEq./L	Ca mg./100 cc.	P mg./100 cc.	Tot. protein Gm./100 cc.	NPN mg./100 cc.	Hematocrit vol. %
				Serum Values					
10–2	103.8	26.0					6.5(RI)		28
10–7							8.6(5.4)		54
10–25	79.0	36.0	141.3	3.1			6.2(2.1)		25
10–26	86.8	34.3			8.9		6.1(3.3)		22
10–27					8.5	3.8	5.8		
10–28	84.8	40+							
10–29					9.3	3.1	6.3		25
10–30	87.0	38.0							
10–31	85.0	37.6				2.5	6.4		18
11–1	91.7	36.0					6.2(3.9)		33
11–2					8.3				26
				Potassium Therapy Started					
11–3	83.9	40+	135.4	2.0		3.0			
11–4	82.7	40+		2.3	9.1				
11–5	80.2	40+	136.6	2.4		2.8			
11–6	87.3	38.5	132.0			3.7	5.6	19.8	19
11–9	84.9	36.8	133.0	3.5	8.4	4.1	5.8		19
11–10	85.8	36.0	132.0	3.5			6.1	23.0	28
11–11	89.9	31.8	134.0	3.5					
11–13	91.4	30.8	136.4	4.2		3.8	6.6	20.8	20
11–15	94.5	28.4	134.6	4.1	10.2	3.7	6.9		21
11–19							6.8		21

therapy was begun, fell even lower to values of 0.5 on October 13. No phosphorus was administered to this patient; "Elamine" contains none.

E.A., J.H.H. #467811, was a 44-year-old white woman who entered the hospital on October 2, 1948. Sixteen years previously an ileostomy had been performed for ulcerative colitis, with rapid improvement in the patient's weight and strength. Aside from the annoyance of the ileostomy, the patient had no complaints. She had been on a general bland diet. Physical examination revealed no important abnormalities. On October 8

a colectomy was performed and the ileum brought down to the anus. A few days after operation, spiking fever and diarrhea set in. The small oral feedings that could be tolerated were supplemented by blood, plasma, glucose, saline and, on two occasions, small amounts of "Amigen"[3] parenterally (Tables 2 and 3 and Fig. 3).

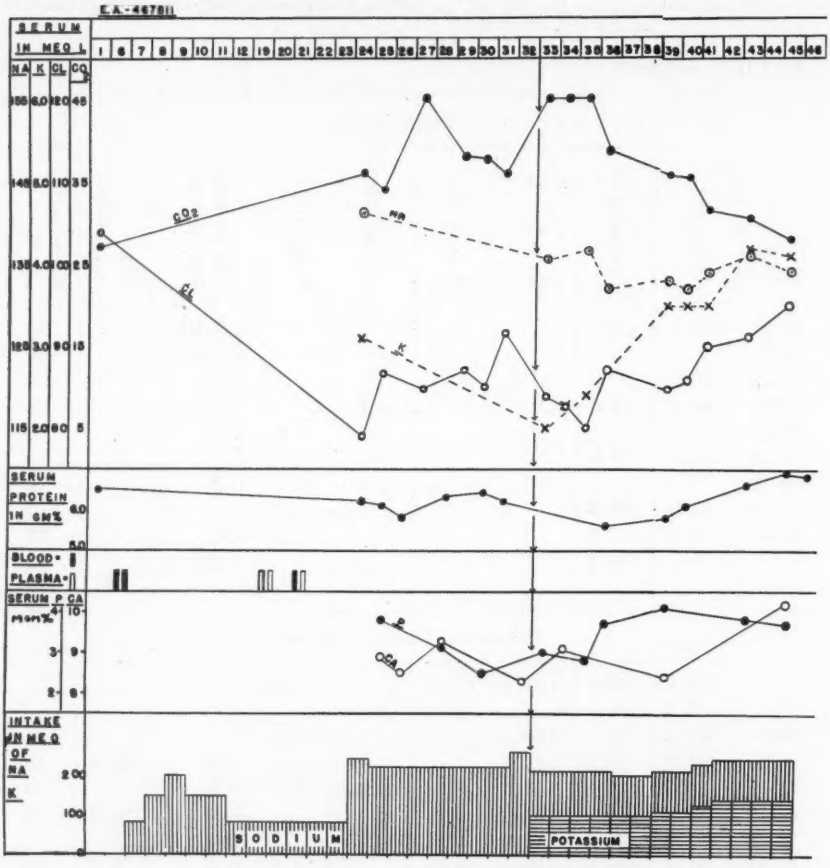

FIG. 3. Electrolyte pattern of serum in case E.A. during 46-day period. The symbols for blood and plasma equal 500 cc.

On November 2 examination in consultation disclosed elevated temperature and pulse, no respiratory distress and normal blood pressure. There was no edema or other evidence of heart failure. The patient was apathetic and weak; peripheral reflexes were practically absent; urine was normal; the cardiogram showed evidences of low serum potassium. The following morning, prior to institution of potassium therapy, the serum concentrations were as follows: potassium 2.0 mEq. per liter, chlorides 83.9 mEq. per

[3] The Amigen used in these studies was furnished by Mead, Johnson & Co., through the courtesy of Dr. Warren Cox, Jr.

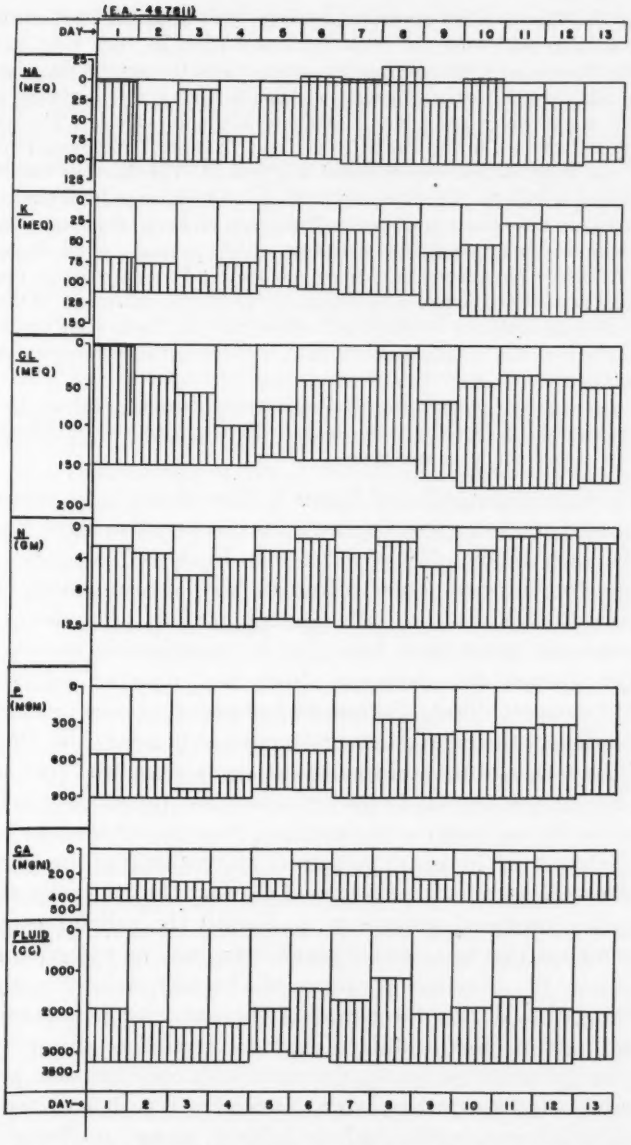

FIG. 4. Balance data of patient E.A. during administration of potassium. Day 1 of this figure is day 33 of Figure 2. Intake is represented by the distance from the lowest line to the zero line. Output is represented by the hatched area. Positive balance is indicated by the clear area below the zero line; negative balance by the hatched lines extending above the zero line.

liter, CO_2 greater than 40 mEq. per liter and sodium 135.4 mEq. per liter; calcium 8.3 and phosphorus 3.0 mg. per 100 cc.

Beginning November 2, oral feeding was stopped and the patient was given by vein each day a mixture of 1 liter of 10 per cent "Amigen," 220 grams of glucose (total fluid volume 3250 cc.) to which was added 8 grams of potassium chloride. The only sodium given was that contained in the liter of 10 per cent "Amigen," approximately 100 mEq. Except for the fever, clinical improvement was prompt. The diarrhea ceased abruptly with instigation of the total intravenous feeding, and drainage from the rectum was negligible until oral feeding was resumed. There was, however, the same delay, as seen in the previous case, in return of the electrolyte pattern toward normal. It was not until November 11 (eight days later) that there was manifest an appreciable rise in serum chloride and a fall in CO_2; and even by November 15 the concentrations of these electrolytes and potassium could not be considered entirely normal. Again there was a coincident appreciable rise in serum protein concentration, without further administration of either blood or plasma.

On November 2, just prior to the total intravenous feeding program, the abdomen was reopened and a perirectal abscess drained. The drainage was considered to be inadequate, and remittent fever continued.

Balance data in Table 2 and Figure 4 show that a large portion of the administered potassium was retained. In the first five days there was a positive balance of 368 mEq. of potassium. In these 2 patients just cited, the analogy to Darrow's observations in his potassium depleted rats is striking. Both patients had suffered long periods of undernutrition in which potassium must have been lost in considerable quantities along with other intracellular elements. Both had received relatively large amounts of sodium chloride. Potassium had probably been further depleted by the diarrhea in the second case. Serum potassium was low, the chlorides low and the CO_2 elevated. Administered potassium was retained in the second case and presumably in the first case also, just as Darrow had found when potassium was given to the depleted rats (25). It was our impression that the delayed rise in serum potassium and reversal of the chlorides and CO_2 concentrations were expressions of the fact that the cells were avidly accepting the proffered potassium; and until the cellular compartment had been repleted up to a certain point, there was no reflection of the improvement in the chemical anatomy of the extracellular compartment. It is striking that during the period of therapy with potassium chloride, the second patient (E.A.) retained chloride throughout the entire period,[4] more in the earlier days when the serum chloride concentration was remaining relatively stationary than later (November 9 to November 13) when serum chloride began to rise. Sodium balance, except on November 5—on which day there is some reason to believe some urine may have been lost—was essentially in equilibrium. It seems likely that the administration of

[4] No attempt was made to allow for sweat in any of these experiments; in none of the patients was undue sweating a feature.

the potassium prevented retention of sodium, which previous experience of our own and that of others would have led us to expect. In E.A. the serum phosphorus concentration remained at all times within normal limits, perhaps because more phosphorus was administered—the 10 per cent "Amigen" solution containing 915 mg. phosphorus per liter. In any event, the over-all balance of phosphorus was strongly positive for the first five days, there being a total of 3,240 mg. phosphorus retained.[5] Comparing the nitrogen retained over the same period (19 grams), it may be seen that nearly three times more phosphorus was retained than the usual ratio of nitrogen to phosphorus in muscle protoplasm; and that the potassium retained was six times as great as the usual potassium to nitrogen ratio in muscle.

EXPERIMENTAL OBSERVATIONS

Other patients, with similar nutritional background, plasma chemical pattern and response to potassium therapy have been observed. One could, however, only guess at the degree of depletion of various elements in such patients, and it seemed wise to turn to animal experimentation in an effort to determine the various factors which might play predominant roles in the production of this type of clinical picture. Last fall, with the help of Dr. H. W. Scott and Mr. Robert Carroll of the Surgical Department, the authors and Miss Marjorie Foote began some observations on dogs. Brief mention of preliminary experiments now in progress follows:

The technique of total intravenous alimentation was used. The apparatus adopted is a modification of that developed by Dr. Rhode (48) of Philadelphia. Fluid mixtures are introduced into the jugular vein continuously. Appropriate bandaging and harness prevent the dog from pulling out the tubing. By an overhead trolley and a series of counterweights the dog may move about the cage or lie down at will without strain on the tubing. Rate of flow is controlled by the usual drop method. By this technique dogs have been kept in a state of good nutrition for as long as 80 days (48).

The first experiments, performed as base-line controls, consisted in giving healthy dogs heavy loads of sodium chloride (2 liters of 0.9 per cent sodium chloride per day) to which 100 grams of glucose had been added. Though the dogs developed obvious edema on the seventh day, the serum potassium did not fall, nonprotein nitrogen and serum sodium did not rise, chloride rose to 122 mEq., CO_2 concentration fell to 15 mEq., serum protein fell from 6.83 to 4.25 Gm. per 100 cc., and there was no conspicuous clinical deterioration of the animals. We were struck by the small amount of potassium lost, a total of 72 mEq. of potassium over the fourteen days,

[5] On November 5, there may have been a portion of urine discarded; even so, the *ratio* between nitrogen and phosphorus and potassium would probably be but little distorted; hence, the figures are given as they stand.

the major portion having been lost on days 1 and 2. These dogs had pylorectomy performed by Dr. Scott on the day of institution of treatment; but since nothing was given by mouth and there was no vomiting, the operation probably contributed no added burden to the animals after the first two days. It was next decided to see what effect exactly the same procedure would have on dogs who had previously been starved for ten days but allowed free access to drinking water. During starvation, urine was collected so that pretreatment losses were known. One such dog was given sodium chloride and glucose only as above; two other dogs were given a mixture consisting of glucose 100 Gm., sodium chloride 2 Gm. and potassium chloride 6 Gm. in 2,000 cc. of water.

After two weeks the dogs given the potassium chloride seemed healthy and frisky; the blood morphology and chemical pattern of the serum were essentially normal. However, on the eighth day the dog given only sodium chloride and glucose was drowsy, apathetic, too weak to stand, slightly edematous and the chemical pattern of the serum was as follows: potassium 2.9, sodium 144, chlorides 114, CO_2 23.2 mEq. per liter, nonprotein nitrogen 27 mg. per cent and hematocrit 46 vol. per cent. The dog was found dead in his cage two hours later.

Analysis of the balance data on these 2 dogs showed the surprising fact that from the beginning of the two types of total intravenous feeding, the over-all negative balance of potassium in the healthy dog (given potassium chloride) was only 150 mEq. less than in the dog that was given sodium chloride and apparently died of hypokalemia. These studies are being continued in an effort to determine the influence of diarrhea, vomiting and other factors in addition to undernutrition on the development of the hypokalemia.

METHOD OF ADMINISTRATION OF POTASSIUM

Despite our admitted ignorance of the exact circumstances leading to potassium deficit, it seems clear that patients are not infrequently met to whom the administration of potassium as a therapeutic agent is advantageous and sometimes a life-saving procedure. When the conditions are suitable for oral administration, obviously this route is to be preferred. A solution of potassium chloride containing 40 to 80 mEq. of potassium per liter (3 to 6 grams of potassium chloride) given in 50 to 100 cc. amounts with or without a fruit juice or some other carrier has seemed entirely efficacious. We have also used one of the meat juice preparations, of which more will be said later, which contains a very high concentration of potassium and which is quite palatable to many persons. But in situations of emergency or when gastro-intestinal dysfunction precludes oral administration, a parenteral route must be used. The plasma reaching the heart must

not contain more than 7 mEq. potassium per liter. This will depend upon not only the concentration of potassium in the administered fluid but also the rate at which it is injected into the vein. Our patients have received solutions containing between 40 and 90 mEq. of potassium per liter at rates not greater than 12 cc. per minute (180 drops per minute). Usually the rate has been about 120 drops per minute (8 cc.). With the more concentrated solutions and rapid infusion rates, under emergency conditions, the cardioscope has been used for quick detection of possible hyperkalemia. As a rule, the potassium salt, kept available in sterile 1 to 3 gram lots, is simply added to whatever infusion mixture seems otherwise most desirable, be it 5 or 10 per cent glucose, a protein hydrolysate or some other solution. Even when so large an amount as 467 mEq. of potassium was given in eighteen hours (43), the concentration of potassium in the infusate did not at any time exceed the figures above, for the amount of fluid injected during the period was 10 liters. Circumstances may arise, as in the presence of heart failure, in which it would be necessary to use a more concentrated solution and less water; it would then be wise to inject the solution at a slower rate.

There have been no personal experiences causing grief with this type of procedure over a period of five years, but there is no doubt that potassium salts should be used with every precaution. A case in point has come to our attention. A patient was clearly suffering with hypokalemia, and the need for potassium was urgent. An overzealous physician injected intravenously within a few minutes a dose of potassium salt which should have been given during an hour or more. While his colleague watched the cardioscope, the picture changed abruptly from that of low potassium to high potassium; and before injection could be stopped, the patient expired.

However, our recent tendencies have been to give larger, rather than smaller, doses. When hypokalemia has appeared in these debilitated types of patients, the receptivity of the cells for the injected potassium has appeared to be very great; and as shown in the preceding graphs, it has usually taken several days to "fill the stockpiles" before the serum potassium has returned to normal levels. In the face of any reduction of urinary volume, it is well to go slowly; and frequent recourse to cardiography or serum potassium determinations is in order.

A word as to the routine use of potassium salts in convalescence. A well-rounded average diet contains, of course, an abundance of potassium, 90–125 mEq. per day. If one can eat beef steak and potatoes, well and good, but in a weakened state after infections or operations, one is apt to turn against even such delectable modern rarities. Perhaps some of the long attested virtue of certain "home" and "folk" remedies lies in their content of potassium. When we were sick as children, our grandmothers would

have a piece of meat or a whole chicken boiled down for us until all the "essence" was out of the meat. This was given to us for its restorative power. As a medical student, J.E.H. scorned the stuff, believing that its sole virtue lay in its sodium chloride content. For many years meat extracts have been given by practitioners to their convalescent patients and taken themselves with satisfaction. Testimonials by these physicians have generally been scoffed at or ignored by the scientific medical world. Broth, as ordinarily prepared for the table, contains approximately 25 mEq. potassium per liter; concentrated stuff such as our home-made meat stock must contain far more. In our laboratory, analysis of a well-known meat juice preparation (Valentine's) showed it to contain 1,215 mEq. potassium to the liter. Four tablespoonfuls of this material contain almost as much potassium as does an ordinary day's diet.

A comment in passing, about the correction of abnormalities in the chemical anatomy of the plasma, when such are met. The usual impulse is to set about correcting all of them as soon as possible. One wonders if this may not sometimes be unwise. There are multiple factors at play which eventually fix the concentration of the various substances in the extracellular compartment. But ultimately the machinery of the kidneys and the cells of the rest of the tissues set the pattern, for the extracellular compartment is inert save for the red blood cells. It seems not unlikely that under circumstances of depletion through illness or inanition, the structure within the cells may be so altered that an environment which we consider "normal" might in reality be very unsuitable. The indiscriminate administration of various materials into the system, be they plasma, sodium, chloride, phosphate or what not, in an effort to restore to the *usual* plasma pattern, may, at times, actually put the cells at a greater disadvantage than that under which they already labor. Perhaps our advocacy of potassium as a therapeutic agent lays us open to a charge of this guilt. But if less concern were wasted on the extracellular environment and more on what this means in terms of the cellular situation and cellular needs, it seems likely that our therapeutic procedures would be more efficacious.

In closing it would be well to return to the initial remarks and repeat that in highlighting the metabolism of a single element, there have been ignored other and equally important intracellular constituents which are integrated in the highly complex intracellular mechanisms. Each of these probably deserves an equal amount of attention. As our knowledge grows, these remarks will doubtless seem inconsequential and immature. They are offered as a temporary glimpse of what will surely become a much broader view, when researches in intermediary metabolism can be more closely correlated with clinical conditions.

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CHANGES IN CIRCULATING EOSINOPHILS IN WOMEN DURING THE MENSTRUAL CYCLE AND REPRODUCTION*

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VARIATIONS in the number of circulating eosinophils were noted as early as 1910 by Dunger (1) who first devised an accurate method for counting these cells. A marked rise in the number of eosinophils is associated with many pathologic conditions. However, a decrease in the eosinophil count has been regarded as of little importance. As an example, the recently published textbook on disorders of the blood by Whitby and Britton (2) attaches no significance to eosinophil counts under 400 per cubic millimeter. However, Schilling (3) described an eosinopenia in the course of acute infections and regarded the failure of the eosinophils to return to the circulating blood as a grave sign.

Renewed interest in circulating eosinophils followed the report of Dougherty and White (4), who demonstrated that pituitary adrenocorticotropic hormone (ACTH) injected into rats produced an increase in the circulating neutrophils and a decrease in lymphocytes. They induced similar changes by the injection of 11-dehydroxy and 17-hydroxy corticosterone. De la Balze, *et al.* (5) found that in patients with Cushing's syndrome there was a leucocytosis and neutrophilia with a relative lymphopenia whereas in patients with Addison's disease there was a relative neutropenia and lymphocytosis.

Forsham and his associates (6) reported that ACTH produces a fall of circulating eosinophils in patients with normal adrenal cortical function. Patients with Addison's disease failed to show this response. These investigators observed that the fall in circulating eosinophils was approximately twice as great as the drop in lymphocytes and, since the difference in response between Addisonian and non-Addisonian individuals was clear-cut, the number of circulating eosinophils is the more sensitive indicator of adrenal cortical function. Thorn and his associates (7) reported that epinephrine reduced the circulating eosinophil count in normal individuals but failed to do so in patients with adrenal cortical failure.

The adrenal gland undoubtedly plays an important role in the repro-

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ductive function. There is ample evidence that adrenal cortical activity is intimately associated with the pituitary and gonads. The demonstration of the relationship between adrenal cortical function and circulating eosinophils prompted this study.

METHOD

White blood cell and eosinophil counts were made from oxalated venous blood. The cells were counted immediately but if this was not feasible, they were stored in the icebox. The oxalate tubes were prepared by the method of Forsham, *et al.* (6), and a Levy counting chamber which has a depth of 0.2 mm. and a ruled area of 16 sq. mm. was used.

Serial determinations were made in as many patients as possible. The number of circulating eosinophils during labor was calculated before the

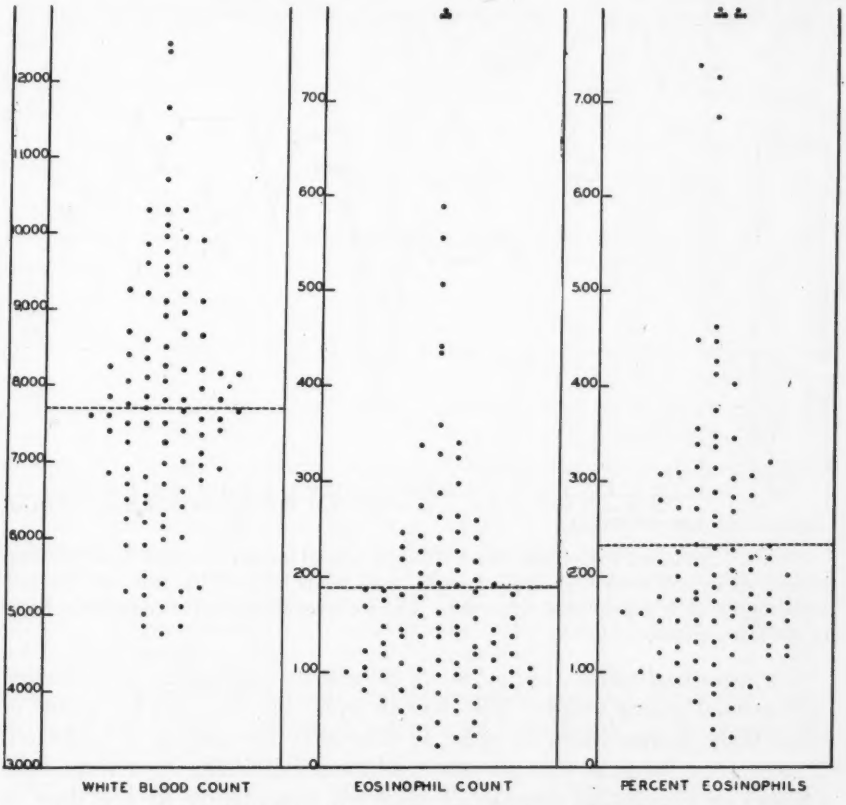

FIG. 1. The total white cell and circulating eosinophil counts in 100 normal young women. The eosinophils averaged 2.3 per cent of the total white count.

onset of contractions early in labor when the cervix was about 5 or 6 centimeters dilated, at the onset of the second stage, and immediately postpartum. Further blood samples were obtained on the first, third, fifth and ninth postpartum days.

RESULTS

Normal women.—Eosinophil counts were made in 100 carefully selected normal individuals. This group was used to establish normal values in women during their reproductive years. There were marked variations in the total white counts among the individuals of this group, but repeated counts in the same individual showed very consistent results. The white count varied from 4,750 to 12,500, within the accepted normal range. The mean percentage of eosinophils was 2.3 (Fig. 1).

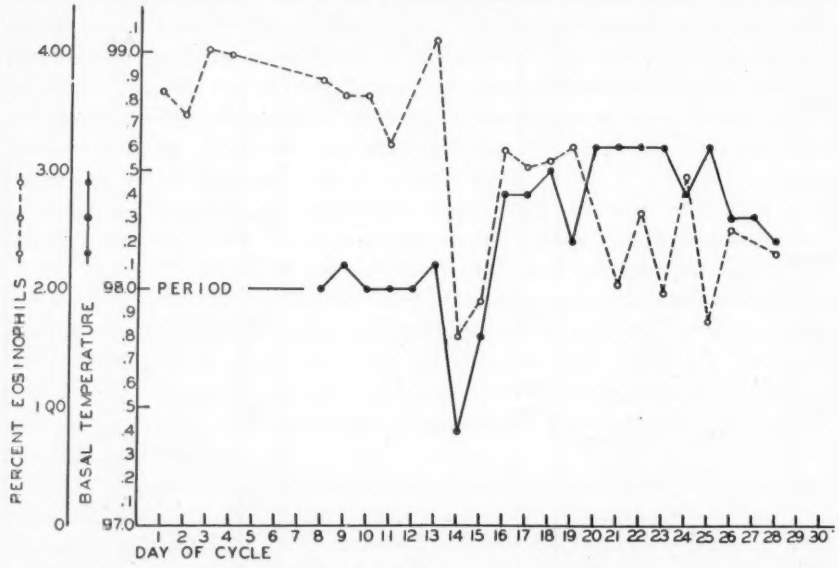

FIG. 2. Circulating eosinophil counts during a typical menstrual cycle. Note the drop in eosinophils about the time that the body basal temperature shifts from the low preovulatory level to the elevated luteal level. There is a tendency for the count to be lower in the luteal phase.

The menstrual cycle.—Variations in circulating eosinophils were studied in 8 normal young women. The ovarian cycle was followed by means of basal body temperatures in order to determine the period of ovulation. Two constant findings were present in all these individuals: 1) a drop in the number of circulating eosinophils occurred consistently at the time of ovulation, as indicated by the rise in body temperature, and 2) the counts were lower during the luteal phase than during the follicle phase (Fig. 2).

Pregnant women.—Determinations were made on 300 normal patients at various periods in their gestations. There was no change in the circulating eosinophil count as the pregnancy advanced. Thus, the period of the gestation did not influence the count. However, a leucocytosis is present in pregnancy. The mean total white count is increased from an average of 7,700 in the nonpregnant individual to a mean of 9,750 in the gravid woman. There is also a relative eosinopenia, the decrease averaging 53

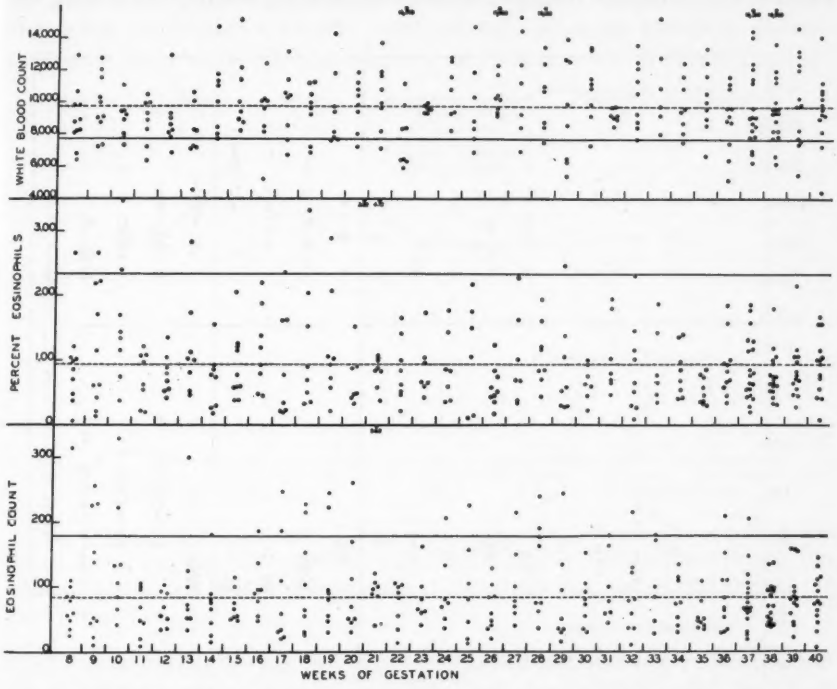

FIG. 3. Total white cell and circulating eosinophil counts in 300 pregnant women throughout gestation. The mean total white count was 9,750 compared with a mean of 7,700 in the nonpregnant control group. There is a relative eosinopenia in pregnancy averaging 0.9 per cent, compared with 2.3 per cent in the control group. However, the eosinophil count remains relatively constant in relation to the total white count, although there is an increasing leucocytosis as pregnancy progresses.

per cent when the nonpregnant and pregnant groups are compared. The calculated percentage of eosinophils decreased from 2.3 for the nonpregnant to 0.9 per cent for the pregnant group (Fig. 3).

The leucocytosis present during pregnancy increases rapidly during labor. The peak of this rising curve is reached in the immediate postpartum period, returning to preparturition levels by the ninth postpartum day.

The leucocytosis of pregnancy has disappeared completely six weeks after delivery.

The most striking change in the number of circulating eosinophils occurs during labor, and consists of an eosinopenia, The first significant manifestation of this change is exhibited during the first stage. The eosinopenia becomes progressively more marked during the second and third stages of labor, reaching an almost total eosinopenia in the immediate postpartum period. The complete disappearance of circulating eosinophils during the

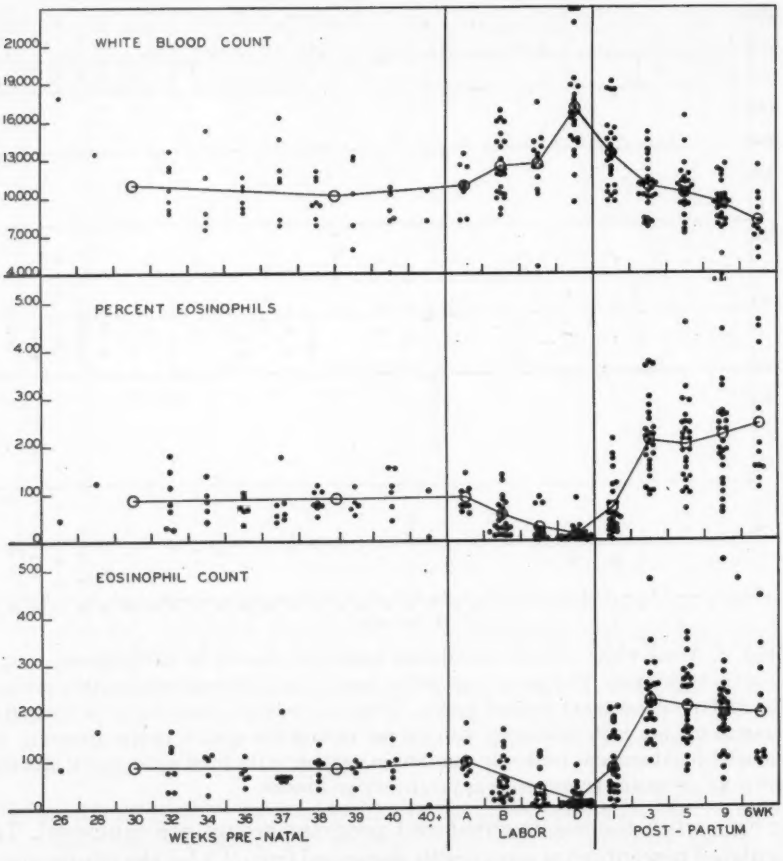

FIG. 4. The total white cell and circulating eosinophil counts of the group of patients followed during pregnancy, labor and the postpartum period. The most striking change is the eosinopenia which develops during labor, reaching a total eosinopenia at the end of labor in more than half the patients. The eosinophil count returns to the normal by the end of the third postpartum day.

final phase of parturition is exceedingly significant. The eosinophil count returns to the normal level by the end of the third postpartum day. The eosinophil response to labor is much more sensitive than the total white count, since the latter has not always returned to the normal nonpregnant level at the ninth postpartum day (Fig. 4).

Eight patients scheduled for elective Cesarean sections under local and general anesthesia were followed by means of eosinophil counts made on the first, third, fifth and ninth postoperative days. These women developed the same eosinophil response observed in the large group of women delivered naturally. The eosinopenia in women delivered abdominally

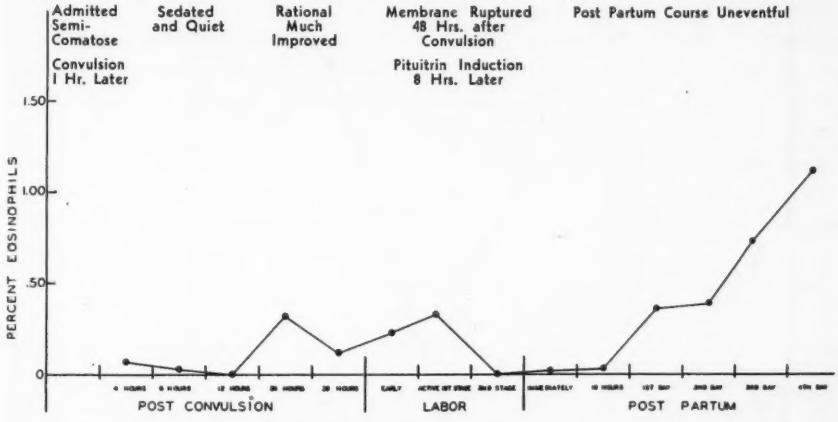

FIG. 5. Eosinophil counts in a patient with eclampsia. Note the almost total eosinopenia during and after the convulsive seizure and during the labor which followed. The return of the normal number of eosinophils to the circulation was delayed beyond the third postpartum day.

lasted a somewhat longer time than in the women delivered through the pelvis. The return to the normal values was postponed until the third postpartum day.

Eclampsia.—An opportunity was provided to study the eosinophil response in this important pregnancy complication in 2 patients. The first patient was not in labor when she had a convulsion. The circulating eosinophils disappeared completely during this seizure. She improved clinically during the next twenty-four hours, and the eosinophils returned to the circulation in small numbers. The induction of labor was followed by their complete disappearance a second time. The return of a normal number of circulating eosinophils following delivery was considerably delayed (Fig. 5).

The second patient had her convulsion during the active first stage of

labor. She was delivered of twins about six hours later. Counts were made one hour after the convulsion, immediately postpartum, and eight hours after her delivery. A total eosinopenia was present throughout this period. The eosinophils returned to the blood stream in appreciable numbers at

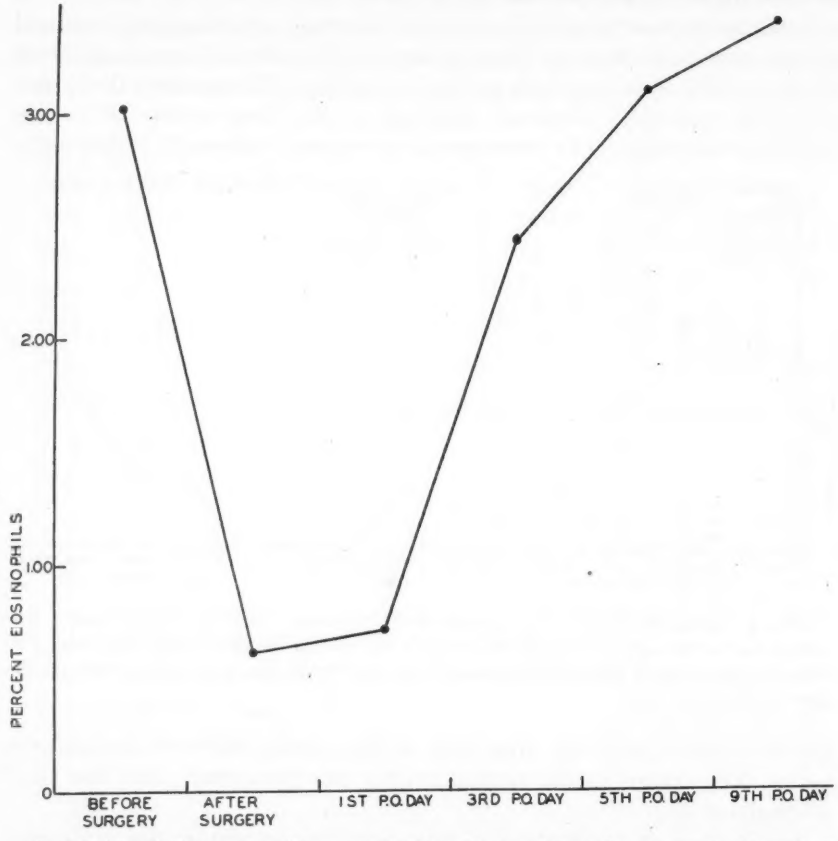

FIG. 6. Circulating eosinophil counts in 10 patients subjected to gynecologic surgical procedures. Note the marked eosinopenia which reached the low level three to six hours after surgery. The count did not return to normal for twenty-four hours.

the end of her first postpartum day, and normal values were reached by the third day.

Surgical operations unrelated to pregnancy.—In order to assess the role of the surgical phase of Cesarean section, 10 patients scheduled for gynecologic surgery, varying from a dilatation and curettage to complete hysterectomy, were studied. Counts were made before, during and after surgery, and on the first, third, fifth and ninth postoperative days.

Preoperative counts were significantly higher than those observed in pregnancy. Following the operation, there occurred a profound drop of the eosinophil count reaching zero in half the patients. The lowest level of the eosinopenia was reached within three to six hours after the operation, and the extent of the drop varied with the magnitude of the surgery. The eosinopenia persisted for about twenty-four hours and the count did not return to the normal until the third postoperative day (Fig. 6).

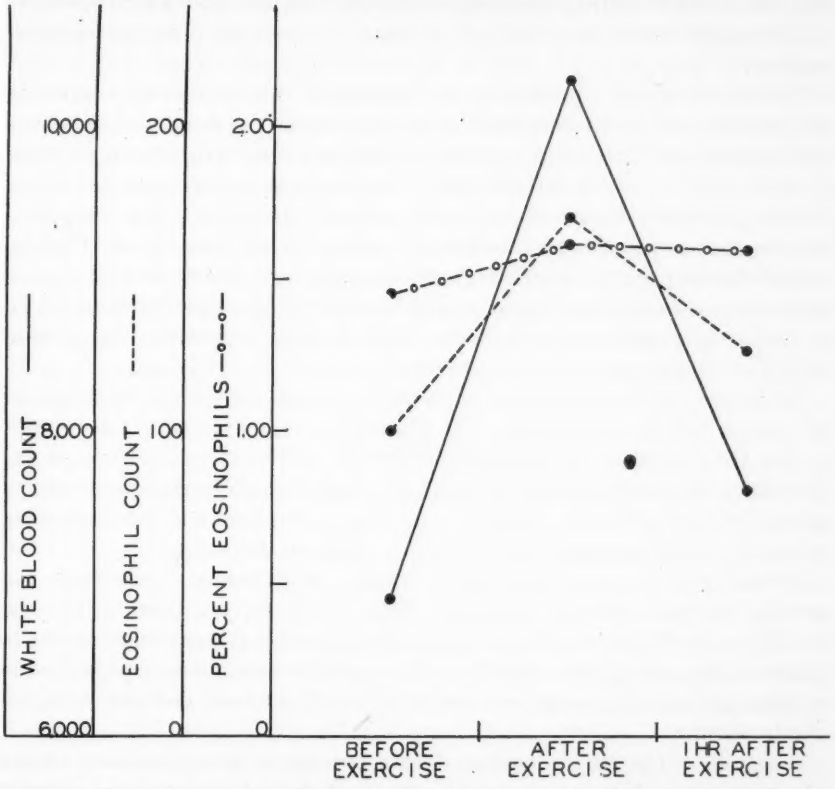

FIG. 7. The influence of moderate physical exertion on the white count and the circulating eosinophils. Moderate exercise results in a rise in the total number of white cells and eosinophils but the percentage of eosinophils remains constant.

Exercise.—The influence of physical exertion on the circulating eosinophils was demonstrated in 4 subjects. They were allowed to run around a square block at a brisk rate. Counts were made before, immediately after, and one hour after this exertion. There was a striking rise in both the total white cell count and the eosinophil count following exercise. The percentage of eosinophils remained relatively constant (Fig. 7).

DISCUSSION

The changes in the circulating eosinophils during the ovarian cycle is noteworthy. The number of cycles followed is too few to draw any accurate conclusions. However, the drop in circulating eosinophils has coincided with the period of ovulation consistently in all the cycles studied and appears significant. There is a tendency for the counts to be lower in the luteal phase than in the follicle phase. It is possible that ovulation itself may be a more profound physiologic stress than we have thought previously. The changes noted may indicate a sensitive gonadal pituitary adrenal balance.

Pregnancy is accompanied by an increase in the number of leucocytes and a decrease in circulating eosinophils. This relative eosinopenia is statistically significant. The stress of labor and delivery results in a rapid decrease of eosinophils reaching an absolute eosinopenia in many patients. These changes probably represent increased adrenal cortical activity, long continued during pregnancy, reaching a climax during parturition. Further studies are in progress which may demonstrate that these changes during reproduction have their origin in increased pituitary activity resulting in an increased production of ACTH, which, in turn, stimulates the adrenal cortex to secrete more cortical steroids.

Eclampsia is a complication of pregnancy that may have its origin in abnormal steroid metabolism. The absolute eosinopenia which developed during the convulsive seizures may represent sudden stress and compensatory adrenal cortical activity, perhaps mediated through the pituitary gland, or it may be an expression of abnormal glandular function. Preeclampsia and eclampsia deserve further study in this light.

Surgical intervention represents trauma, and the eosinopenia is the natural adrenal cortical response (Selye's "alarm reaction" (8)). The sensitiveness of the reaction is extremely interesting. Anesthesia seems to influence the eosinopenia very little, for similar responses were provoked in surgical procedures carried out under a variety of local and general anesthetic agents.

The role of the adrenal cortex during periods of stress has been known for many years. Numerous experimental and clinical observations indicate that the adrenal cortex secretes 11-oxysteroids as a protective mechanism during prolonged physical exertion, anoxia, exposure to cold or trauma, starvation and infection. These cortical steriods can be measured. Furthermore, adrenalectomized animals and patients with adrenal failure have a decreased tolerance to stress which can be increased by the administration of adrenal cortical extracts.

Thorn and his co-workers (7) demonstrated that the injection of ACTH

offered a method of ascertaining the function of the adrenal cortex. In the normal subject, the injection of ACTH is followed by a prompt fall in the number of circulating eosinophils and a rise in the acid-creatinine ratio in the urine. These effects are negligible in the presence of adrenal cortical failure. Recant, Forsham and Thorn (9) extended the usefulness of this observation by noting that the administration of epinephrine to normal animals produced an increase in adrenal cortical activity, but this response was absent in the hypophysectomized animal. This action presumably stems from the stimulation of pituitary function by the epinephrine and the subsequent release of ACTH, which in turn stimulates adrenal cortical activity. Thus, relatively simple methods are available not only for the detection of primary pituitary and adrenal disease, but they may be applicable in uncovering transient adrenal impairment as a result of surgical or obstetrical procedures.

These studies reveal that labor produces a period of physiologic stress which is easily combatted by normal adrenal function. Serious complications of pregnancy, such as eclampsia, produce more marked changes. It is possible that some of the previously unexplained states of shock in pregnancy and labor may well have their origin in transient pituitary or adrenal cortical failures. Their diagnosis and treatment should be facilitated by our new knowledge.

SUMMARY

The numbers of circulating eosinophils were determined in normal women during the various phases of the ovarian cycle, during pregnancy, delivery and the postpartum period, during and following Cesarean section and gynecologic operative procedures in eclampsia and following moderate physical exertion. A correlation of circulating eosinophils and the ovarian cycle was demonstrated. Pregnancy results in a leucocytosis and a relative eosinopenia. Labor is associated with an increasing eosinopenia so that delivery is often climaxed by a total absence of circulating eosinophils. Eclampsia and surgical procedures are characterized by a marked or absolute eosinopenia. The significance of these physiologic changes is discussed.

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THE USE OF THE HUMAN VAGINAL SMEAR IN THE ASSAY OF ESTROGENS*

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LABORATORY assay methods have proven inadequate to determine the relative clinical potency of the various estrogens. By rat assay, the apparent potency of estrone varies with the number of injections and the vehicle in which the hormone is injected. Laqueur (1) found the rat unit of estrone equivalent to 10 to 16 international units, but Rowe and Simond (2) obtained a value of only 3 or 4 international units. Estradiol benzoate is approximately ten times as potent as estrone when assayed in the rat, but only four times as potent (Laqueur) or of equal activity (Rowe and Simond (3)) when tested in the mouse. The outstanding example of misleading laboratory assay is the discrepancy between rat assays and clinical trials of the methyl ether of bis-dehyrdo-doisynolic acid. In the rat, this compound when given subcutaneously is more active than estradiol, whereas, clinically it is practically inert (Segaloff (4, 5)).

Since 1941 many studies on the amounts of various estrogens necessary to relieve subjective menopausal symptoms have appeared, but relatively few authors have used objective criteria to compare different estrogens, or the same estrogen by different routes. Mack and Ale (6) by daily vaginal smears from a group of women demonstrated that estrone would restore glycogen to the vaginal epithelium. The smallest dose employed was 5 mg. There was no appreciable difference in effect, except that the oral dose produced a less sustained response. Stoddard and Metzger (7) studied vaginal smears taken twice weekly before, during and after ten-day treatment intervals. By intramuscular administration, estradiol benzoate was only twice as effective as estrone. Furthermore 0.4 mg. estradiol benzoate intramuscularly was equivalent to 0.5 mg. diethylstilbestrol given orally.

Castrodale, Loeffel and MacBryde (8) found that stilbestrol was no

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more effective sublingually than orally on the basis of vaginal smear changes. They concluded that stilbestrol was twice as effective when given intramuscularly (0.5 mg. being equivalent to 1.0 mg. orally) in this "human assay." Shorr (9) reviewed the advantages of vaginal smear studies and made some comparisons of orally administered estrogens. He noted an equivalent response to ethinyl estradiol, 0.2 mg.; diethylstilbestrol, 2.0 mg.; and monomethylstilbestrol 4.0 mg., when these doses were given daily for an unstated interval. He also found that 2.0 mg. of stilbestrol orally was equivalent to 0.5 mg. estradiol benzoate intramuscularly. Allen (10) determined the minimal doses that would induce bleeding in castrate women. On the basis of this endometrial response, he found that an oral dose of ethinyl estradiol, 0.4 mg., was equivalent to 3.0 mg. of stilbestrol. This ratio of 1 to 7.5 compares favorably with Shorr's ratio of 1 to 10. Allen also determined that stilbestrol, 3.0 mg., orally, was equivalent to 1.0 mg. of estradiol benzoate intramuscularly. This 3:1 ratio approximates the 4 to 1 value of Shorr but differs from the 1.0 to 0.8 findings of Stoddard and Metzger.

Ryden (11) compared the estrogen effect in 8 castrate women, using weekly endometrial biopsies, and concluded that Dinestrol injected intramuscularly was 5.7 times as effective as when given orally. Dinestrol and estradiol benzoate were equivalent in potency when given intramuscularly.

The purpose of this study was to determine the minimal effective dose of various estrogens as revealed by changes in the vaginal smears of postmenopausal women.

MATERIAL AND METHODS

Estrogens available on the market as well as some special preparations were used. When given orally, they were administered daily for ten days, following the pattern set by Stoddard and Metzger. Intramuscular preparations were given as single injections. Stilbestrol tablets were purchased from the local pharmacy. Crystalline stilbestrol furnished by Smith-Dorsey was made up in sesame oil (5 mg./cc.) and so diluted that each test dose was given in 1 cc. of oil. Estrone (Theelin-In-Oil) was furnished by Parke, Davis & Co. in ampoules of 10,000 I.U. (Lot No. 3165625) for intramuscular use, and in gelatin capsules containing 5,000 I.U., for oral administration. The mixture of estrone and estriol (Theelin and Theelol, 0.5 mg. of each in oil per gelatin capsule), estriol (Theelol, 0.25 mg. per Kapseal, Lot No. F326F), and the mixture of natural estrogens (Menagen x1947 in tablets containing 10,000 I.U.) were also furnished by Parke, Davis & Co. An aqueous suspension of crystals and an oil solution of mixed estrogens (Estrogenic Substances) were furnished by Smith-Dorsey. Aqueous suspensions of large and small crystals (RB 134–44 and RB 134–43) of natural

estrogens and oily solutions of trimethyl estradiol acetate (Estrotate, Lot No. RB 11–1D), in concentrations of 0.33, 0.50, 1.0 and 1.66 mg./cc. were furnished by Lakeside Laboratories.

The subjects were postmenopausal women housed in the Iowa State Hospitals and were selected because they showed atrophic vaginal smears. Groups of at least 10 and usually 15 to 20 women were given the same dose of an estrogen and the degree and duration of vaginal responses were noted. The women were given a large "priming" dose of estrogen (5 mg. of stilbestrol or 4 mg. of a natural estrogen), and when the smears had regressed to their original stage, usually after four to six weeks, a test dose was given. When this test dose produced a definite response it was reduced one-half and the next trial made after the vaginal smears had regressed again. In this procedure, the large doses sufficed to "prime" the subject for the subsequent reduced dose. When a dose was reached which induced a very minor response or none at all, it was concluded that the minimal effective dose had been determined.

Smears were obtained weekly by inserting a moist cotton swab into the vagina. Then the swab was rolled over a slide and the smear allowed to dry in the air. These were examined as dry, unstained preparations under a low power objective.

Smears were recorded in four grades: *Grade 1*, an atrophic stage with only small basal epithelial cells and leukocytes. *Grade 2*, predominantly intermediate cells characterized by large, round cells with large, vesicular nuclei. These cells may contain considerable glycogen as demonstrated by the Mack iodine vapor stain method. *Grade 3*, early stages of cornification with many leukocytes. *Grade 4*, fully cornified smear with very few leukocytes.

EXPERIMENTAL

An oral dose of 2.5 mg. diethylstilbestrol per day for ten days increased the average grade of the vaginal smears of 22 women from 2 to 3.5 and then it regressed in the next three weeks (Fig. 1). One milligram orally per day produced very similar results but 0.5 mg. per day produced only half the rise in smear grading, and this was judged as a minimal dose.

Diethylstilbestrol was then administered intramuscularly to 19 women. A single injection of 5.0 mg. produced a marked response and it was five weeks before the vaginal smears returned to their initial stage (Fig. 2). A single dose of 2.0 mg. gave nearly as great a response, whereas 1.0 mg. produced definite but minor responses and 0.5 mg. was ineffective.

From these findings, it appears that a single intramuscular dose of 1.0 mg. of stilbestrol produces a response similar to that induced by 1.0 mg. orally per day for ten days. Possibly this ratio of 1:10 is not a fair com-

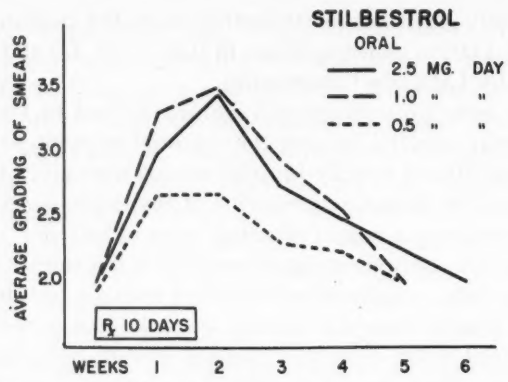

FIG. 1. Graphic representation of the vaginal smear changes induced by various doses of stilbestrol given orally for ten days. These observations were successive tests made on the same group of patients.

parison due to difference in duration of administration of the estrogen by the two routes. Castrodale, Loeffel and MacBryde found the potency of the oral dose to be only twice that of the intramuscular when daily doses were administered in each instance.

Estrone (Theelin-In-Oil) given orally to 16 women in doses of 2.0 mg. and 1.0 mg. daily produced equally marked changes in the average smear grading (Fig. 3). Again, as after stilbestrol, five or six weeks elapsed before

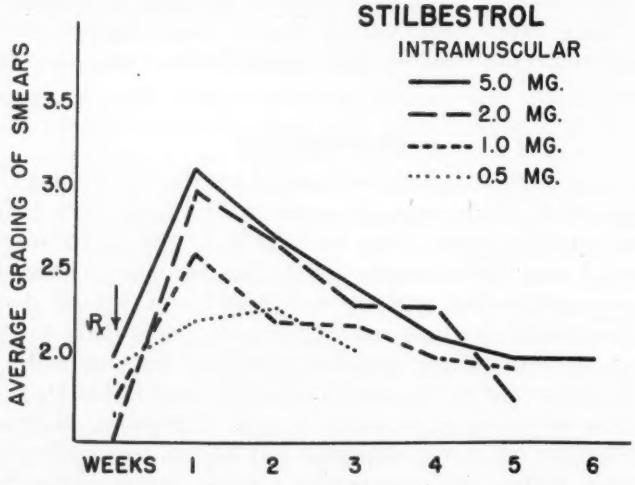

FIG. 2. Graphic representation of the vaginal smear changes induced by an intramuscular injection of stilbestrol in 1 cc. of oil. These observations were made on 12 patients remaining from the original group of 19 who were given stilbestrol orally.

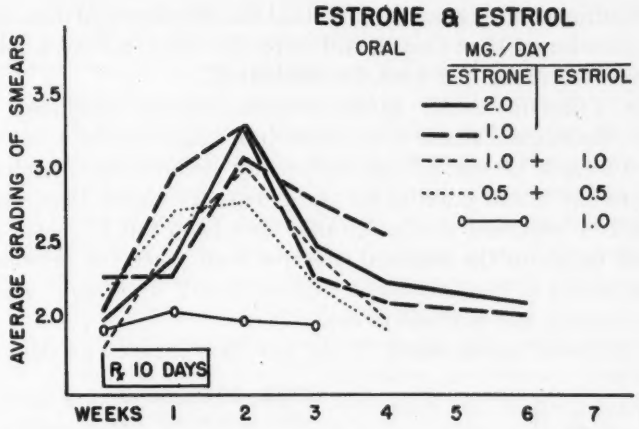

FIG. 3. Graphic representation of the vaginal smear changes resulting from the oral administration of estrone, estriol, and estrone with estriol for ten days (Theelin and Theelol-Parke, Davis & Co.). The two tests with the 1 mg. dose of estrone are plotted for comparison. At these dose levels, estriol was ineffective by itself and had no additive effective when given together with estrone.

the smears regressed to their former grades. When 0.5 mg. was given, the response was neither as great nor as prolonged, so that 0.5 mg. appeared to be about the minimal effective dose. These findings confirm those of Mack and Ale that estrone is effective when given orally. Furthermore, estrone appears to be as effective as stilbestrol, milligram for milligram, when given orally. These findings on changes in vaginal smears are in marked contrast to those of Allen, based on minimal doses required to

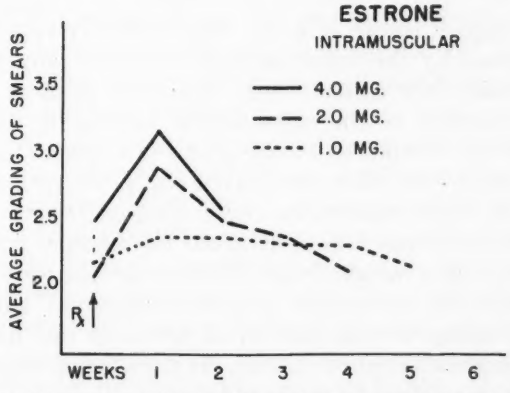

FIG. 4. Graphic representation of the vaginal smear changes following an intramuscular injection of estrone (Theelin-In-Oil).

induce withdrawal bleeding. He found that the effective oral dose of estrone was ten times larger than that of stilbestrol (31.5 mg. per week for estrone as compared to 3.0 mg. per week for stilbestrol).

Estrone (Theelin-In-Oil) given intramuscularly produced marked changes in the vaginal smear when given in a single dose of 4 mg. or 2 mg. (Fig. 4). A dose of 1.0 mg. did not induce any distinct rise in vaginal cornification but the initial grading for the group was higher than usual. The test should be repeated at the 1.0 mg. dose level but it seems probable that it will be about the minimal effective dose, since the response to 1.0 mg. of stilbestrol is very similar to that of 2.0 mg. of estrone, whereas 0.5 mg. of stilbestrol was without effect.

Estriol (Theelol) given orally (1 mg. per day) induced no demonstrable

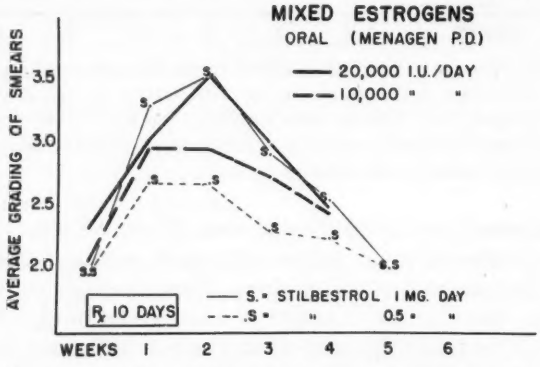

FIG. 5. Graphic representation of the vaginal smear changes resulting from the oral administration of natural estrogens (Menagen) for ten days. The curves showing the response to stilbestrol are transferred from Figure 1.

effect on the vaginal smear (Fig. 3). This ineffectiveness of estriol was further manifested by the failure of estriol to exert any additive effect over that obtained with estrone alone. This lack of effect of oral estriol is in marked contrast to the experimental findings in rats. Rowe and Simond found that it required 5 micrograms of estrone (15 subcutaneous rat units) to produce an effect when given orally, whereas only 3.0 micrograms of estriol (3 subcutaneous rat units) induced an estrous smear when given orally. On a rat-unit basis, estriol was five times as potent as estrone for oral use but on a weight basis (3.0 micrograms were equivalent to 5.0 micrograms) the advantage was more apparent than real. Even this slight advantage of oral potency of estriol in rats does not appear to have any clinical application, 1.0 mg. of estriol being ineffective, whereas 1.0 mg. of estrone produced a maximal response.

The mixture of natural estrogens occurring in pregnant mare's urine

(approximately 90 per cent estrone) was tried in several forms by oral and intramuscular routes. An oral preparation (Menagen) was given at two dose levels. The change in vaginal smear grading in 11 women given 2 capsules (20,000 I.U.) daily was similar to that induced by 0.5 mg. of stilbestrol (Fig. 5). In this instance, it is not possible to make a weight comparison. However, it has been shown that stilbestrol subcutaneously by rat assay is about 2.5 times as potent as estrone (25,000 I.U. as compared to 10,000 I.U. per mg.). On this basis, the human response to oral administration of these two substances shows a good correlation with their respective estrogenic potencies in international-unit equivalents.

The natural mixed estrogens were given intramuscularly as aqueous suspensions of crystals and as oily solutions. An aqueous suspension of crystals (Smith-Dorsey) was given at two dose levels, 40,000 I.U. and 20,000

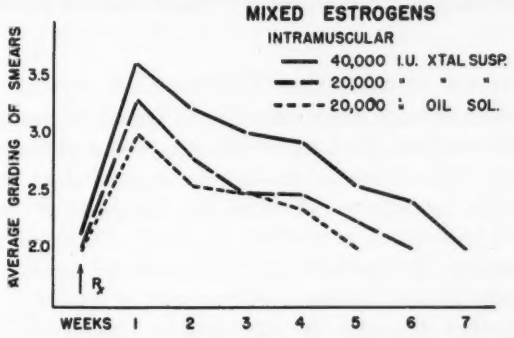

FIG. 6. Graphic representation of the vaginal smear changes induced by an intramuscular injection of natural estrogens (Smith-Dorsey) as aqueous suspensions of crystals and in oily solution.

I.U. The larger dose produced a somewhat greater and more prolonged response (Fig. 6) but there is little appreciable difference in degree or duration of response from 20,000 I.U. as crystals, compared to the same dose in an oily solution (Smith-Dorsey). By comparison, the responses to 2.0 mg. (20,000 I.U.) of estrone and 1.0 mg. of stilbestrol (25,000 I.U.) were almost identical to that of 20,000 I.U. of natural mixed estrogens in oil. Here again the effectiveness of the natural estrogens and stilbestrol were comparable on the basis of international-unit equivalents.

Two sizes of crystals were used to determine whether larger crystals might prolong the effectiveness. Aqueous suspensions of large crystals and small crystals were prepared by Lakeside Laboratories. There was no appreciable difference in effect when given in 20,000 I.U. doses (Fig. 7). The average measurements of these crystals in microns (large 10.62×7.7, small 8.7×4.0) were determined by Prof. V. W. Meloche, University of

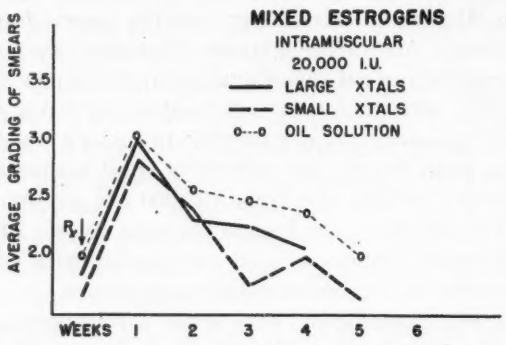

FIG. 7. Graphic representation of the vaginal smear changes induced by an intramuscular injection of aqueous suspensions of large and small crystals of natural estrogens (Lakeside Laboratories). The curve showing response to an oil solution is transferred from Figure 6.

Wisconsin. It seems probable that even the large crystals were so small that the relative difference in size was not sufficient to change their rate of absorption appreciably since their effect was not prolonged over that of an oily solution. Both sizes would have been graded as fine crystals by Miescher, Gasche, and Frey (12) who list less than 50 microns as fine crystals, 50 to 150 microns as medium and 150 to 250 microns as coarse. They also found that flat platelet crystals are more quickly absorbed due to their relatively greater surface area per unit of volume.

The most potent estrogen encountered in this study was trimethyl estradiol acetate (Estrotate-Lakeside). The dose was reduced from an initial 3.3 mg. to 0.5 mg. without any marked decrease in response (Fig. 8). A dose as low as 0.16 mg. still produced an effect greater than that following

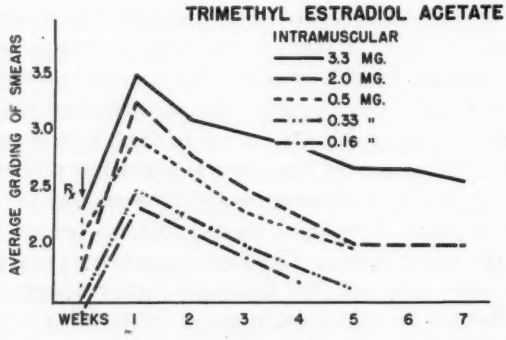

FIG. 8. Graphic representation of the vaginal smear changes following an injection of trimethyl estradiol acetate (Estrotate-Lakeside).

0.5 mg. of stilbestrol. Thus by intramuscular injection, the trimethyl ester of estradiol acetate appears to be at least three times as potent as stilbestrol in stimulating the vaginal epithelium of women.

An unsuccessful attempt was made to study estrogen withdrawal bleeding. The subjects of this study were mental patients and their history of such bleeding was unreliable. The comments of the attendants were likewise found to be unreliable. Though on many occasions blood was found on the vaginal smear, it was felt that the single weekly contact with these women did not give satisfactory data as to the incidence, amount, or duration of bleeding.

Bleeding did occur in some members of each group when large doses were used and it is our impression that this sign was more frequent when oral medication was employed. Further studies on more cooperative subjects will be necessary to determine the uterine threshold, or withdrawal-bleeding requirements of these estrogens.

DISCUSSION

The reasons for the apparent differences in potency of estrogens in different species probably will be found in metabolic studies. The mouse seems unable to split the benzoate off the estradiol ester as readily as the rat. The ability of the rat liver to inactivate estrogens may be greater than that of the human (13).

Hooker, Drill and Pfieffer (14) found that pellets of estrogen implanted in the spleen of the monkey were as effective as those implanted subcutaneously. This is in marked contrast to the inactivation of estrogens injected into the spleen of rats (Segaloff). Clinical studies designed to test the relative effectiveness of estrone and of estradiol when given orally or intramuscularly in single or in divided doses are necessary before the effect of the liver can be assessed. The fact that estriol is a form of estrogen peculiar to the human (it is excreted in the urine of pregnant women and has not been found in other species) makes it possible to rationalize species difference in response to this substance. Estriol should be tried in doses of 5.0 or possibly 10.0 mg. per day to learn whether this excreted substance has any estrogenic potency in women. The estrogenic action of the synthetic nonsteroid substances will be an enigma until their metabolic breakdown is known.

SUMMARY

The species variation in response to various estrogens is so great that clinical evaluation of these substances is dependent upon trial in human subjects. When compared in postmenopausal women on the basis of the vaginal smear response it was found that:

1. Estrone given orally in daily doses for ten days is as effective as stilbestrol.
2. Estrone given intramuscularly in a single dose is about one-half as potent as stilbestrol.
3. Estriol in the oral dosage used (1 mg. per day) had no demonstrable estrogenic effect, whereas estrone was effective.
4. Natural estrogens (approximately 90 per cent estrone) are effective orally and compare favorably with stilbestrol on an international-unit basis.
5. Suspensions of crystalline estrogens do not have any apparent depot effect when the crystal dimensions are no greater than 10 microns.
6. Estradiol (trimethyl acetate) produced maximal response in doses of 0.5 mg. intramuscularly and there was a minimal response following 0.16 mg. intramuscularly.

Addendum

Since this article was written, assays of trimethyl estradiol acetate by the oral method have demonstrated maximal vaginal response in doses of 5.0 mg., 2.0 mg., and 1.0 mg. daily for ten days, and considerable activity in a dose of 0.5 mg. daily.

Acknowledgments

We are indebted to Drs. Soucek and Ristine of the Mount Pleasant State Hospital for making patients available for this study.

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PROGESTERONE: A COMPARISON OF INTRAMUSCULAR, ORAL AND SUBLINGUAL ROUTES OF ADMINISTRATION*

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THE widespread use of progesterone in combination with estrogen in the cyclic steroid therapy of menstrual irregularities, together with its empirical use in the treatment of threatened and habitual abortion, makes urgent the need for effective progestational hormones which may be administered other than by injection.

It is generally believed that progesterone itself is inactive when administered by the oral route to human patients. This belief appears to be based on several studies in animals. A search of the literature has failed to disclose that the oral efficacy of progesterone in human patients has ever actually been adequately studied. It was apparently on the basis of the animal studies that anhydrohydroxyprogesterone (Pranone, Progestoral, Lutocylol) was synthesized and is now exclusively used as the only effective nonparenteral type of progesterone therapy.

In view of this, it was decided to investigate the sublingual and oral administration of progesterone itself in human patients. Actually the sublingual administration to human subjects of free progesterone has already been studied by Greenblatt (1). In 21 amenorrheic women the dose necessary to cause withdrawal bleeding was found to be from 125 to 150 mg. of progesterone. In the same study Greenblatt found the effective sublingual dose of anhydrohydroxyprogesterone to be 100 to 125 mg.—not a very striking difference between the two compounds.

METHODS

Patients with secondary amenorrhea associated with a persistent proliferative-phase endometrium were test subjects for this study. They varied in age between 19 and 27 years.

A type of Corner-Allen progesterone test used for assaying progesterone in animals, was employed with these patients. They were primed with estrogen (4,000 I.U. Urestrin[1] per day orally) for twenty days. On the last five days of the estrogen treatment they were then given the progesterone

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[1] Mixed natural estrogen (Upjohn).

preparation. The progestational effect was determined by the amount of withdrawal bleeding and by endometrial biopsy.

RESULTS

Intramuscular progesterone

Prior to initiating this study or at some time during the study, it was shown that each of these 18 patients was capable of responding to intramuscular progesterone by the development of a progestational endometrium. The degree of endometrial response was variable, some responding with a well defined secretory endometrium to 5 mg. of progesterone daily for five days, whereas one patient required a minimum of 10 mg. daily for nine days to produce the slightest endometrial response. A fair to good secretory response invariably followed a dose of 10 mg. daily for five days when preceded by oral estrogen over a period of fifteen to twenty days. In all cases the progestational response was improved by the preliminary administration of estrogen.

Withdrawal bleeding occurred in every case primed with estrogen followed by five days of progesterone administration, but only 4 of 9 patients tested had withdrawal bleeding after progesterone alone.

Daily subcutaneous or intramuscular administration of progesterone in a dose of 10 mg. daily for five days usually induces progestational changes on the sixth day from the onset of treatment. Larger doses than 10 mg. do not seem to accelerate nor potentiate the progestational response and a dose less than 10 mg. per day for five days is followed by variations in the degree of progestational changes. With the knowledge that 10 mg. of progesterone intramuscularly daily for five days is approximately the minimal effective dose when given after priming with estrogen, it was adopted as the base line against which to project the results obtained from oral and sublingual administration.

Sublingual progesterone

Using patients from the preceding group after a rest period of at least three weeks since receiving intramuscular progesterone and a minimum of seven weeks of amenorrhea, there were 12 patients with secondary amenorrhea or oligomenorrhea of a functional nature treated by sublingual progesterone alone (Table 1). The hormone was administered to these patients in doses varying from 5 to 8 linguets daily for five days. Each linguet contained 10 mg. of pure progesterone, and the total of 50 to 80 mg. was given in divided doses three times daily. None of these patients had uterine bleeding within seventy-two hours after completing five days of sublingual progesterone therapy. There were 5 patients studied in 7 cycles of treatment who received the hormone in a dose of 50 mg. daily in divided doses for

five days and of these, 2 had very scanty bleeding lasting less than an hour. Endometrial biopsy specimens taken twenty-four hours after completing treatment showed early progestational changes in 1 and no effect in 6 of the treatment cycles. There were 9 cycles in which 80 mg. daily in divided doses was given for five days and 1 of these was followed by four hours of uterine bleeding, not sufficient to require a vulva pad. Biopsy specimens taken within twenty-four hours on 4, and within forty-eight hours on 5, showed transitional endometrium in 5 and full progestational effect in 4.

TABLE 1. SUBLINGUAL PROGESTERONE THERAPY IN PATIENTS PRIMED WITH ESTROGEN (URESTRIN), 4,000 I.U. ORALLY PER DAY FOR 20 DAYS.

Dose of progesterone per day for last 5 days of estrogen treatment (mg.)	No. of cycles	Withdrawal bleeding	Endometrial biopsy
50	7	2 cycles, scanty bleeding	1 cycle, early progestational 6 cycles, no effect
	9	8 cycles, normal amount and duration 1 cycle, 4 hours' duration, scanty	5 cycles, transitional endometrium 4 cycles, full progestational effect
90	6	5 cycles, normal amount and duration 1 cycle, 8-hour period	1 cycle, full secretory effect 5 cycles, progestational effect

There were 6 cycles in which 90 mg. daily in divided doses was given for five days and in 1 of these a bleeding episode lasting about eight hours occurred. Endometrial biopsy on the day after completing the five-day treatment course was compared with the biopsy prior to the onset of treatment in the patient who had the bleeding response, and it was found that the endometrium was converted from a persistent proliferative type into a well developed secretory type. The other 5 biopsies showed evidence of progestational effect.

There was 1 patient (M2182), aged 31, who had a secondary amenorrhea of eighteen months' duration. During the first year of amenorrhea, cyclic uterine bleeding was induced with stilbestrol-progesterone, stilbestrol 5 mg. daily for twenty days and progesterone subcutaneously in a dose of 10 mg. on the fifteenth, twentieth and twenty-fifth days of the cycle. This treatment was discontinued and the patient lapsed into a period of six months' amenorrhea. She was then given Urestrin capsules (1 daily for

twenty days) and progesterone linguets (100 mg. daily in a divided dose on the last five days of Urestrin administration). She had free bleeding three days after completing this twenty-day treatment and endometrial biopsy revealed a well developed secretory phase with marked vascularity and gland lumens filled with secretion. The same patient was treated during the succeeding month with progesterone linguets alone. Linguets in a dose of 50 mg. daily were administered in a divided dose for five days; withdrawal bleeding did not occur and the endometrium was of the proliferative type.

There were 2 other patients with amenorrhea of 6 and 8 months' duration respectively. Treatment with Urestrin capsules (1 daily for twenty days) and progesterone linguets (50 mg. daily for the last five days of Urestrin administration in a divided dose) was followed by withdrawal bleeding in both of these patients. Their endometriums were proliferative in type.

TABLE 2. ORAL ANHYDROHYDROXYPROGESTERONE THERAPY IN PATIENTS PRIMED WITH ESTROGEN (URESTRIN), 4,000 I.U. ORALLY PER DAY FOR 20 DAYS.

Dose of anhydrohydroxy progesterone per day for last 5 days of estrogen treatment (mg.)	No. of cycles	Withdrawal bleeding	Endometrial biopsy
80	8	Occurred in 8 cycles	No evidence of progestational effect
130	6	Withdrawal bleeding in 5 No bleeding in 1	2, good progestational effect 4, moderate progestational effect

When these same patients were given progesterone linguets alone, no bleeding occurred and no endometrial response could be seen. They were then treated by Urestrin daily for twenty days and progesterone linguets 140 mg. daily in a divided dose on the last five days of estrogen treatment. Withdrawal bleeding occurred in both cases from a fully developed progestational endometrium.

Oral anhydrohydroxyprogesterone

The same 14 patients from the original group with secondary amenorrhea and oligomenorrhea who were available for comparative study with orally administered anhydrohydroxyprogesterone were used (Table 2). Since it had already been shown that the minimum effective dose of the hormone sublingually was approximately 80 mg. per day when administered in

conjunction with oral estrogen in the form of Urestrin, it was decided for purposes of comparison to start these patients on 80 mg. of anhydrohydroxyprogesterone in a divided dose daily for five days, in conjunction with Urestrin. This was done, endometrial biopsy specimens being taken before treatment and on the day following the completion of the five-day treatment. There was withdrawal bleeding in 8 patients, but a study of the endometriums showed no evidence of stimulation. Six of these patients were then studied during the oral administration of Urestrin daily for twenty days and 130 mg. of anhydrohydroxyprogesterone daily for five days on the last five days of Urestrin administration. Withdrawal bleeding occurred in 5, free bleeding in 4. Two patients who showed the maximum degree of uterine bleeding had a well developed secretory phase endometrium; lesser progestational effect could be seen in the other tissues studied. Three of these patients were then subjected to the same treatment schedule except that the anhydrohydroxyprogesterone dose was increased to 250 mg. per day for five days. Endometrial response was excellent. It was concluded therefore that 80 mg. of sublingual progesterone was approximately equivalent to 130 mg. of oral pregneninolone when used in conjunction with oral estrogen therapy for the induction of uterine bleeding in amenorrheic patients. Six patients with secondary amenorrhea varying in duration for eight to twenty-four weeks were treated by the oral administration of 150 mg. of anhydrohydroxyprogesterone with the simultaneous administration of estrogen. Uterine bleeding occurred in 5 of these patients, a transitional type of endometrium was found in 1, and a normal progestational endometrium in 4.

There was 1 patient with amenorrhea of twenty months' duration in whom withdrawal bleeding had previously been produced by other treatment. She was given anhydrohydroxyprogesterone in a dose of 80 mg. in a divided dose daily for five days with no bleeding and no endometrial response. She was then given the same drug orally in a dose of 110 mg. daily, with no response. A third course of treatment with 140 mg. daily for five days was not followed by bleeding, but there was evidence of progestational activity on endometrial biopsy.

Oral progesterone

A comparison of sublingual progesterone tablets placed in the oral cavity until dissolved was made with the same tablet swallowed (Table 3). Nine patients from the original group were available for study and 6 of them cooperated through the two treatment cycles. Progesterone was swallowed in a divided dose of 80 mg. daily for five days in 6 patients without previous priming with estrogen. There was no withdrawal bleeding, but endometrial biopsy revealed a transitional type in 3 and a fair progestation-

TABLE 3. ORAL PROGESTERONE. (NO PRIMING WITH ESTROGEN)

Dose	No. of cycles	Withdrawal bleeding	Endometrial biopsy
80 mg. per day for 5 days (Progesterone)	6	No bleeding	3 cycles, transitional endometrium 3 cycles, fair progestational effect
80 mg. per day for 5 days (Anhydrohydroxy progesterone)	9	—	No progestational response

al type in 3. The same 9 patients were then given anhydrohydroxyprogesterone in a dose of 80 mg. daily; there was no endometrial response evident in any biopsy specimen studied. It had previously been shown in 5 of this group that a dose of 130 mg. of anhydrohydroxyprogesterone was the minimal effective dose for inducing progestational endometrium.

DISCUSSION

The studies on the effectiveness of the sublingual administration of progesterone to human patients reported here confirm and extend those o Greenblatt (1,2). In addition to the withdrawal bleeding employed by Greenblatt, another objective criterion, endometrial biopsy, was used. Since the withdrawal bleeding in these studies could have been on the basis of estrogen withdrawal, it is felt that the progestational effect observed in the endometrial biopsies is of greater significance.

The observation of greatest importance in this investigation is that the results indicate that progesterone itself is active when administered by the oral (swallowed) route. Furthermore, progesterone itself appears to be more active orally than anhydrohydroxyprogesterone. This is completely contrary to what has been believed to be true for man. As far as animal studies are concerned, the reports to date are controversial. Thus, Miescher and Gasche (2) found progesterone to be only one-sixtieth as active in rabbits as anhydrohydroxyprogesterone when administered orally. Soderwall (3), on the other hand, reported that progesterone was more active orally in inducing sexual receptivity in spayed female guinea pigs. One mg. of progesterone orally induced heat in 84.6 per cent of the animals, whereas 1.0 mg. of anhydrohydroxyprogesterone orally induced heat in only 62.5 per cent.

In view of the fact that our observation that progesterone is more active orally than anhydrohydroxyprogesterone is contrary to the accepted belief,

conjunction with oral estrogen in the form of Urestrin, it was decided for purposes of comparison to start these patients on 80 mg. of anhydrohydroxyprogesterone in a divided dose daily for five days, in conjunction with Urestrin. This was done, endometrial biopsy specimens being taken before treatment and on the day following the completion of the five-day treatment. There was withdrawal bleeding in 8 patients, but a study of the endometriums showed no evidence of stimulation. Six of these patients were then studied during the oral administration of Urestrin daily for twenty days and 130 mg. of anhydrohydroxyprogesterone daily for five days on the last five days of Urestrin administration. Withdrawal bleeding occurred in 5, free bleeding in 4. Two patients who showed the maximum degree of uterine bleeding had a well developed secretory phase endometrium; lesser progestational effect could be seen in the other tissues studied. Three of these patients were then subjected to the same treatment schedule except that the anhydrohydroxyprogesterone dose was increased to 250 mg. per day for five days. Endometrial response was excellent. It was concluded therefore that 80 mg. of sublingual progesterone was approximately equivalent to 130 mg. of oral pregneninolone when used in conjunction with oral estrogen therapy for the induction of uterine bleeding in amenorrheic patients. Six patients with secondary amenorrhea varying in duration for eight to twenty-four weeks were treated by the oral administration of 150 mg. of anhydrohydroxyprogesterone with the simultaneous administration of estrogen. Uterine bleeding occurred in 5 of these patients, a transitional type of endometrium was found in 1, and a normal progestational endometrium in 4.

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The studies on the effectiveness of the sublingual administration of progesterone to human patients reported here confirm and extend those o Greenblatt (1,2). In addition to the withdrawal bleeding employed by Greenblatt, another objective criterion, endometrial biopsy, was used. Since the withdrawal bleeding in these studies could have been on the basis of estrogen withdrawal, it is felt that the progestational effect observed in the endometrial biopsies is of greater significance.

The observation of greatest importance in this investigation is that the results indicate that progesterone itself is active when administered by the oral (swallowed) route. Furthermore, progesterone itself appears to be more active orally than anhydrohydroxyprogesterone. This is completely contrary to what has been believed to be true for man. As far as animal studies are concerned, the reports to date are controversial. Thus, Miescher and Gasche (2) found progesterone to be only one-sixtieth as active in rabbits as anhydrohydroxyprogesterone when administered orally. Soderwall (3), on the other hand, reported that progesterone was more active orally in inducing sexual receptivity in spayed female guinea pigs. One mg. of progesterone orally induced heat in 84.6 per cent of the animals, whereas 1.0 mg. of anhydrohydroxyprogesterone orally induced heat in only 62.5 per cent.

In view of the fact that our observation that progesterone is more active orally than anhydrohydroxyprogesterone is contrary to the accepted belief,

further studies are now being conducted to confirm this preliminary report. These will provide a basis for a subsequent paper.

SUMMARY

1. Progesterone administered intramuscularly will consistently induce a progestational endometrium in the estrogen-primed woman after a dose of 10.0 mg. daily for five days.

2. Progesterone administered sublingually is effective in producing a progestational effect on the endometrium when given in a dosage of 80 mg. per day for five days.

3. Progesterone administered orally will produce a moderate progestational effect when given in a dose of 80 mg. daily for five days.

4. Anhydrohydroxyprogesterone administered orally produces a progestional effect on the endometrium in about 50 per cent of estrogen-primed patients who receive 80.0 mg. daily for five days.

5. Preliminary administration of estrogen is necessary for the full progesterone effect, whether the progesterone be administered intramuscularly, sublingually, or orally.

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THE EFFECT OF ALPHA-TOCOPHEROL ADMINISTRATION ON PREGNANEDIOL EXCRETION*

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VITAMIN E (alpha-tocopherol) has been widely used clinically in the treatment of disorders of reproduction since shortly after its discovery by Evans and Bishop (1) in 1922. It has been clearly demonstrated by animal experimentation that a vitamin E deficiency is associated with sterility in the male and resorption of embryos in the female rat. Any rationale for vitamin E therapy in related disorders of the human, however, has been lacking. The concept of a human diet deficient in vitamin E is difficult to accept and until recently no reliable, simple method was available for determining the vitamin E status of an individual.

Winkler (2, 3) and Winkler and Bach (4) have reported that the administration of vitamin E increases the ovarian and pituitary function in women. The increased function has been demonstrated by measuring the cyclic pregnanediol, estrogen and F.S.H. excretion of subjects before and during alpha-tocopherol administration. Stohler and Kaiser (5) in 1941 presented some experimental evidence for a similar effect in the rat. Such a finding is theoretically attractive, especially from the standpoint of estrogen and progesterone metabolism. If the anti-oxidant properties of vitamin E protect these unsaturated sterols from rapid oxidative destruction, the result might be an increase in the activity of these hormones. A sound basis for vitamin E therapy in some cases of sterility and habitual abortion would thus be established.

As only six feeding experiments with concomitant hormone excretion curves have been reported, it was deemed advisable to attempt to extend this work. Only the relationship of vitamin E administration to corpus luteum function has been investigated in the present study. Three normal laboratory workers volunteered for the experiment and seven feeding experiments were run on these individuals, all of whom were having regular menstrual cycles. Two additional feeding experiments were run on patients complaining of sterility but having regular ovulatory cycles as determined by endometrial biopsy. Two additional cycles on a sixth subject have been included for purposes of comparison. Serum tocopherol levels were deter-

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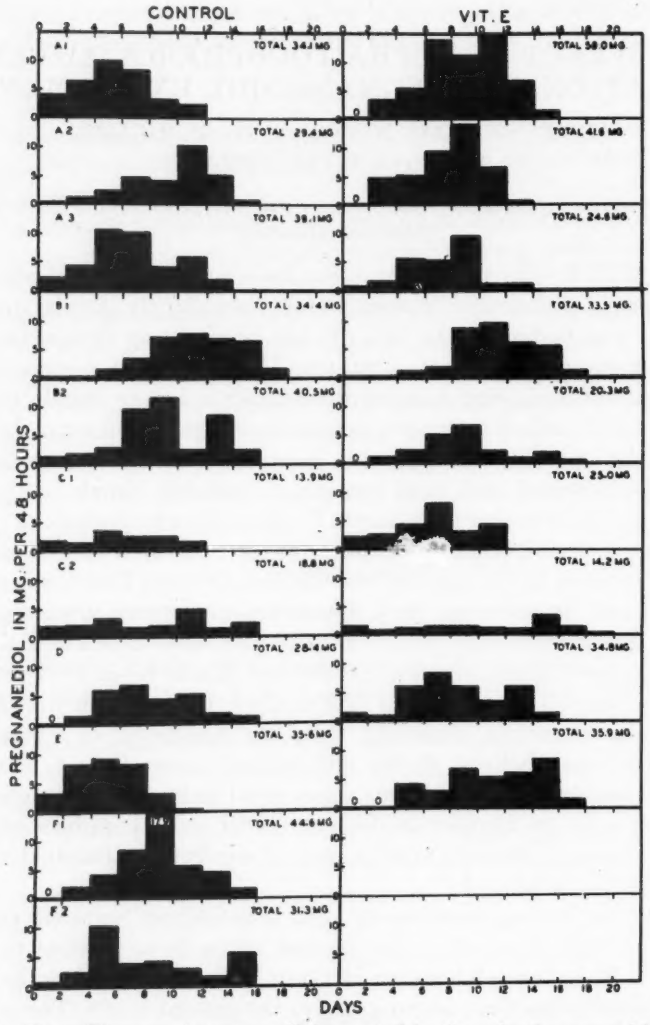

FIG. 1. The milligrams of pregnanediol excreted in the urine by normal individuals during 11 untreated menstrual cycles are compared with the amounts of pregnanediol excreted during the following menstrual cycles by the same individuals when alpha-tocopherol was administered. Pregnanediol is expressed as milligrams excreted per 48 hours, day 1 being the estimated day of ovulation.

mined before and during vitamin E administration on several subjects. Basal temperature charts on one individual were used as an aid in the analysis of luteal function.

METHODS

The pregnanediol excretion has been determined by the modified Astwood-Jones (6) technique, using 48-hour specimens in every case. The method is one which was devised in this laboratory and has been used constantly during the past eight years. Recovery of amounts of pregnanediol of less than one milligram for a 48-hour excretion period are considered unreliable and have been regarded as zero in the present calculations. Recovery of one milligram amounts has been consistent and such values are therefore considered accurate. Every precaution is taken in technique to make the determinations quantitative.

The serum vitamin E levels were measured by a modification of the Quaife-Harris method (7).

EXPERIMENTAL

Cyclic pregnanediol excretion for 5 individuals before and after vitamin E administration is shown in Figure 1. In addition, two normal cycles on a sixth individual have been charted.

There are three control cycles and three cycles during vitamin E administration shown on the first normal individual (A—1, 2 and 3). There is an increase of the total excretion from 34.1 mg. for the first control month, to 58.0 mg. for the month during which 50 mg. a day of vitamin E was administered. The following control month showed a total pregnanediol excretion of 29.4 mg., whereas the next cyclic excretion increased to 41.6 mg. when vitamin E in the same amount was again administered. The third feeding experiment was done on this subject four years after the original experiment and this time the total pregnanediol excretion in the control cycle amounted to 39.1 mg. When 50 mg. a day of vitamin E was administered, the excretion of pregnanediol, instead of rising, fell to 24.6 mg. The serum tocopherol before administration was 1.08 mg. per cent and following administration, 1.24 mg. per cent, both values being well within normal limits for the method used (8).

The next normal subject, B, showed a total urinary pregnanediol excretion of 34.4 mg. during a control cycle and 33.5 mg. when 50 mg. of vitamin E was taken daily. The second feeding experiment on this subject showed a total pregnanediol excretion of 40.5 mg. for the normal cycle and 20.3 mg. for the vitamin E administration cycle. It may be noted that these values were obtained at the same time that those of A1 and A2 were determined.

The third normal individual also ran two control and two vitamin E administration cycles (C1 and C2). The first control cycle showed a total of 13.9 mg. of pregnanediol excretion increasing to a total of 25 mg. when 50 mg. of vitamin E was taken daily. The second control cycle three years later showed a total of 18.8 mg. of pregnanediol, whereas the output during the vitamin E administration cycle was only 14.2 mg. The temperature

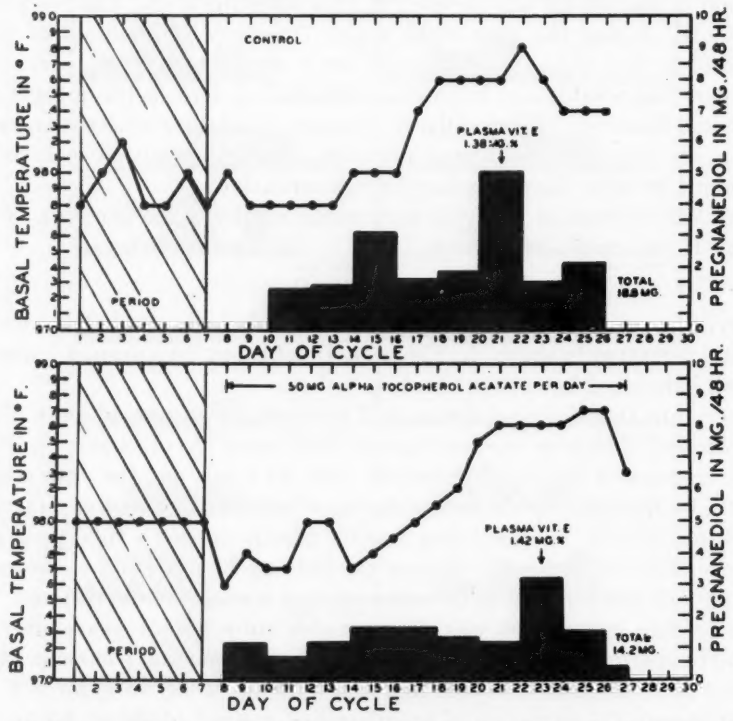

FIG. 2. Pregnanediol excretion blocked in milligrams for 48 hours is shown in relation to the day of the menstrual cycle and the basal body temperature. The first cycle illustrated is the control and the second is the menstrual cycle during which 50 milligrams of alpha-tocopherol was given each day.

charts obtained concomitantly with these pregnanediol excretion values are shown in Figure 2. The serum tocopherol level in this subject was 1.38 mg. per cent on November 26, 1947, at the height of the luteal phase in the control cycle. On December 7, immediately before vitamin E administration, the value was 1.07 mg. per cent and on December 23 during vitamin E administration the value was 1.42 mg. per cent, all within normal limits.

The fourth subject, D, who presented a sterility problem, had a total

pregnanediol excretion of 28.4 mg. in her control cycle with an increase to 34.8 mg. during the following month when 150 mg. a day of vitamin E was administered.

The fifth individual, E, was also a sterility problem. This patient had a total pregnanediol excretion of 35.6 mg. during her control month and the excretion remained at 35.9 mg. during the following month when she was given 50 mg. of vitamin E daily.

The final two cycles, F1 and F2 were normal control cycles (from the same individual). The first month, this individual excreted 44.6 mg. and the following month, a total of 31.3 mg. of pregnanediol.

DISCUSSION

Three cycles in the present study showed an increase in the amount of pregnanediol excreted during the vitamin E administration period over the amount of pregnanediol excreted during the control cycle (A1, A2, C1), two showed a decrease (A3, B2) and four showed no significant variation (B1, C2, D, E). The variations are further minimized when comparisons are made between the untreated cycles of the same individuals. Control cycles of subject A showed a total pregnanediol output of 31.4, 29.4 and 39.1 mg. Subject B showed somewhat less variation (34.4 mg. and 40.5 mg.) and subject C showed little variation (13.9 and 18.8 mg.). Subject F excreted 44.6 mg. in one cycle and 31.3 mg. in another, the difference of 13.3 mg. being the greatest variation in the present small series. In the light of these observations only one vitamin E feeding cycle (A1) could possibly be considered as increased over the normal variation, with an increase of 23.9 mg. Since a vitamin E feeding cycle in another individual, (A2) showed a decrease of 20.2 mg. rather than an increase, it would seem that even these relatively wide variations must be considered as perhaps due to some normal physiologic variation of corpus luteum function. This is in accord with the findings of Venning and Browne (9) and others.

The duration of the luteal phase also showed no constant variation during vitamin E administration. Four cycles were apparently increased (A3, C2, D, E), four were similar (A1, B1, B2, C1) and one (A2) was decreased in length over the normal control cycle. The variation in the duration of all the luteal phases was from a minimum of 12 days to a maximum of 18 days. The figure 18 was obtained as an estimation with the use of the temperature chart (Fig. 2). The duration of the menstrual cycles varied from a minimum of 23 days to a maximum of 32 days. The longest luteal phase, one of 18 days, occurred in a menstrual cycle lasting but 27 days, whereas the shortest of 12 occurred in a cycle of 24 days' duration.

Analysis of the material presented indicates that there is no constant

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effect upon the corpus luteum function of normal individuals when as much as 150 mg. a day of alpha-tocopherol is given throughout the menstrual cycle. From the present experiment it is not possible to say whether abnormal individuals or individuals with a vitamin E deficiency, as indicated by the serum levels, would respond differently to the administration of alpha-tocopherol or whether a longer period of medication would lead to different results.

SUMMARY

1. The urinary pregnanediol excretion during 20 menstrual cycles of 6 apparently normal individuals has been studied. Eleven were control cycles and 9 were during periods of vitamin E administration.

2. Alpha-tocopherol serum levels were obtained on 2 of the subjects before and during vitamin E administration. These levels were within the normal range.

3. There is apparently a wide variation in the normal amount of pregnanediol excreted during the menstrual cycles of the same individual and a wider normal variation of excretion from individual to individual. No regular variation in pregnanediol excretion was noted when alpha-tocopherol was administered in doses of 50 to 150 mg. per day for one month.

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THE TRANSPORT AND EXCRETION OF URIC ACID IN MAN. V. A SEX DIFFERENCE IN URATE METABOLISM

*WITH A NOTE ON CLINICAL AND LABORATORY FINDINGS IN GOUTY WOMEN**

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RECENT studies have tended to confirm the long suspected importance of the sex endocrines in gout (1, 2, 3, 4). Smyth, Cotterman and Freyberg (1) found that male relatives of gout patients who inherit asymptomatic hyperuricemia do not usually develop abnormally elevated plasma urate levels until after puberty. Stecher, Hersh, and Solomon (2) demonstrated that female carriers of this gene generally do not develop abnormally elevated plasma urate levels until the menopause, or the period of failing ovarian function which precedes the menopause. Because some years of hyperuricemia generally precede the onset of clinical symptoms, clinical gout follows a similar pattern, but at an interval of some years. Clinical gout in men seldom appears before puberty. In women symptoms ordinarily first occur after the menopause. Exceptionally, women do develop gout well before the menopause, possibly because of special hereditary factors (*see* addendum regarding gout in women). Hill (5) pointed out that in women with premenopausal gout, attacks of acute gouty arthritis tended to occur at the menses, the low point of estrogen secretion in the menstrual cycle.[1] Since the time of Hippocrates, gout has been reputed to be particu-

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[1] Attempts to treat gout with estrogens have apparently met with only indifferent success. We have seen no striking clinical change in two women with tophaceous gout and in one man with tophaceous gout who received rather large doses of stilbestrol. However, in a patient with pseudohermaphroditism and adrenogenital syndrome (Table 4), very large doses of stilbestrol appeared to cause some fall in plasma urate. Coste (6) has also

larly infrequent in eunuchs, but this question does not appear to have been studied critically.

A relative increase in androgen/estrogen ratio would seem to favor the appearance of asymptomatic hyperuricemia in an individual predisposed by heredity, and hence also to favor the later development of clinical gout. A qualitative alteration in androgen metabolism may also be important in the mechanism of gout. Wolfson and associates (4) reported very low outputs of urinary 17-ketosteroids in all of 11 gout patients studied. They were able to rule out all usual endocrine causes of this finding and to show that gout patients were able to transform exogenously introduced testosterone to ketosteroid at the usual rate. Because the hypogonadism ordinarily expected to accompany very low 17-ketosteroid outputs actually did not occur, they suggested that biologic androgen activity in gout was maintained by an abnormal androgen which, when metabolized, did not contribute importantly to urinary 17-ketosteroids. Since either men or women can inherit gout, they assumed the abnormal androgen to originate in the adrenal cortex. A second convergent series of recent studies has suggested participation of the pituitary-adrenocortical mechanism in attacks of acute gouty arthritis and in the regulation of urate metabolism (9–19).

Such observations lend interest to claims that the average plasma urate level of normal adult men is somewhat higher than that of normal adult women. Our data again confirm the existence of this sex differential and show that it occurs in the gouty as well as in normal adults. Results of renal clearance studies permit some preliminary insight into the mechanism by which this differential is maintained. The possible relationship of the normal sex differential to the mechanism of inherited hyperuricemia in gout will be discussed below.

METHODS AND SUBJECTS

Control subjects were normal young adults and ambulatory medical patients with minor ailments. The gout patients were research patients of the Department of Biochemistry and patients of the Arthritis Clinic. Unless otherwise stated, the urate values given are colorimetric total urate values rather than true urate values, as in our other clinical publications.

In carrying out studies of urate metabolism or excretion, it is important to avoid administering foreign substances whenever possible, since urate clearance is notoriously labile to pharmacologic influences. For this reason, glomerular filtration rates were estimated as $(1.75) \times$(urea clearance) with urine flows maintained constant or decreasing, and between absolute

been unable to influence gout with female sex hormones. Marañon (7, 8) on the other hand, has suggested intermittent estrogen therapy in gout, but has published no data on the results of such therapy.

urine flow limits of 12.0 and 2.0 ml. per minute. When this precaution is observed, the glomerular filtration rates estimated by this indirect procedure have been found to check well with inulin clearances or with endogenous creatinine clearances.

RESULTS

Table 1 compares the distribution of individual plasma urate values and the average plasma urate levels of the two sexes in four groups of

TABLE 1. DATA INDICATING A SEX DIFFERENTIAL IN PLASMA URATE CONCENTRATION BETWEEN MALE AND FEMALE NORMAL ADULTS AND BETWEEN MALE AND FEMALE GOUT PATIENTS

(Values for gout patients were determined while patients were on self-selection diets, without salicylate or cinchophen medication. Because more male than female patients were excluded from the table owing to uricosuric medication, the patients listed below are not a complete cross section of our gout series, so far as sex distribution is concerned.

Data on normal children are taken from a study conducted in cooperation with Dr. David Krevsky on urate metabolism in children. Note the absence of a sex differential in children.)

Range of plasma urate	Number of subjects							
	Normal children		Normal adults		Pretophaceous gout patients		Tophaceous gout patients	
mg. per 100 ml.	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
0.6 through 1.5	—	1	—	—	—	—	—	—
1.6 through 2.5	4	5	—	—	—	—	—	—
2.6 through 3.5	10	11	—	5	—	—	—	—
3.6 through 4.5	8	9	2	11	—	—	—	—
4.6 through 5.5	1	1	14	4	—	1	—	—
5.6 through 6.5	1	—	6	—	—	1	—	—
6.6 through 7.5	—	—	—	—	4	3	—	1
7.6 through 8.5	—	—	—	—	12	2	—	—
8.6 through 9.5	—	—	—	—	5	—	1	—
9.6 through 10.5	—	—	—	—	2	—	3	1
10.6 through 11.5	—	—	—	—	1	—	4	—
11.6 through 12.5	—	—	—	—	—	—	2	—
12.6 through 13.5	—	—	—	—	—	—	—	—
13.6 through 14.5	—	—	—	—	—	—	—	1
Average plasma urate mg. per 100 ml.	*3.39*	*3.21*	*5.27*	*4.05*	*8.10*	*6.70*	*11.05**	*10.20**
Sex differential in plasma urate, mg. per 100 ml.	*0.18*		*1.20*		*1.60*		*0.85*	

* Median values.

TABLE 2. RATIO OF AVERAGE FEMALE TO MALE PLASMA URATE CONCENTRATIONS IN REPORTED STUDIES ON NORMAL ADULTS

(No distinction has been made between plasma and serum, both being included under plasma analyses. The term "Folin-Benedict" refers to color development technique which involves a phosphotungstate or arsenophosphotungstate color development with cyanide intensification. In all cases, a "direct" technique was used without previous isolation of urate as the silver salt. The method of Leone (23, 24) involves spectrophotometric determination of urate without previous deproteinization; all other techniques involve deproteinization.)

Authors	Material analyzed	Color development	Uricase	Normal men		Normal women		*Female/Male* Ratio
				No.	Plasma urate, mg.%	No.	Plasma urate, mg.%	
Present report	Plasma	Folin-Benedict	No	22	5.27	20	4.05	0.77
Jacobson (20)	Plasma	Folin-Benedict	No	63	4.40	37	4.00	0.91
Mull (21)	Plasma	Folin-Benedict	No	51	4.30	22*	3.81	0.89
Mull (21)	Plasma	Folin-Benedict	Yes	51	3.23	22*	2.30	0.71
Bulger and Johns (22)	Plasma	Ferricyanide	Yes	62	4.40	41	3.40	0.77
Leone (23, 24)	Plasma	Folin-Benedict	Yes	59	5.81	49	5.17	0.89
Stecher *et al.* (3)	Plasma	Folin-Benedict	No	396	3.95	294	3.63	0.92
Brøchner-Mortensen (25)	Plasma	Ferricyanide†	No	25	7.62	25	6.35	0.83
Berglund and Frisk (26)	Blood	Folin-Benedict	No	43	3.20	89	2.70	0.84
Brown (27)	Blood	Folin-Benedict	No	12	2.84	17	2.39	0.84

Median *Female/Male* ratio in all series summarized above: *0.84*

* In 37 normal pregnant women in the last trimester of pregnancy, the plasma total urate (no uricase) value averaged 3.59 mg.% and the true urate values (uricase), 2.24 mg.%. These values are 94% and 97% respectively of the values for normal nonpregnant woment (21).

† Brøchner-Mortensen's technique is generally believed to give values which are somewhat too high.

subjects: normal children, normal adults, patients with pretophaceous gout, and patients with tophaceous gout. In each group, except children, men have higher average urate levels than women, although there is considerable overlap in the distributions.

Tophi are usually a late development in gout and perhaps represent a complication rather than an integral part of the disease. Plasma urate levels in tophaceous gout tend to be considerably higher than in pretophaceous gout, but this may be due to the greater degree of renal impairment in patients with tophi. Fifteen of our patients with pretophaceous gout have been found to have an average glomerular filtration rate of 76 cc./min./1.73 sq.M., whereas 5 patients with tophaceous gout had an average of only 44 cc./min./1.73 sq.M.

Because of this somewhat equivocal status of the subcutaneous tophus, a better measure of the constitutional factor which produces gouty hyperuricemia is the magnitude of the difference in plasma urate concentration between the average patient with pretophaceous gout and the average normal adult of the same sex. In our series, this difference, calculated from the data of Table 1, is 2.83 mg. per cent in men and 2.65 mg. per cent in women. In other words, the genetic factor appears to produce an approximately equal elevation of plasma urate level in both sexes, when the norm is taken as the average adult plasma urate concentration of normal individuals of the same sex.

Table 2 summarizes the results of a number of studies in which urate levels of normal men and women have been compared. All recent studies show the existence of the sex differential. The average ratio of female/male plasma urate levels appears to be about 0.84. Two factors appear to explain the rarity of clinical hyperuricemia in casual groups of adult females. The more important factor is the lack of recognition of the normal sex differential; but an additional cause must be the failure to recognize that inherited hyperuricemia in women is not usually completely manifested until after the menopause (3). There is, in addition, some suggestion that urate levels are higher in postmenopausal nongouty women than in premenopausal women (3).

The only large series of comparative data on gouty men and women is that of Hill (5). In 59 men with typical articular gout, Hill found that 44 per cent had blood urate concentrations above 5.5 mg. per cent, whereas only 25 per cent of 16 gouty women had similarly elevated levels. In a number of published series which have included a few gouty women, the average blood urate concentrations of the women have been less than those of the men. This is true of the reports of Brøchner-Mortensen (25), Talbott (28), Stecher (29), Herrick and Tyson (30) and Gibson and Kersley (31).

Table 3 summarizes clearance studies on gouty and control subjects.

The ratio of urate clearance to glomerular filtration rate is somewhat higher in the women than in the men of either group. Analysis of the mechanism is better carried out by reference to the control group in order to avoid alterations secondary to renal impairment. In the control group, the urate clearance is somewhat greater in the women than in the men, whereas glomerular filtration rate is somewhat greater in the men. Both differences operate toward making the ratio of urate clearance to glomerular filtration rate lower in men.

TABLE 3. AVERAGE URATE CLEARANCE DATA IN CONTROL AND GOUTY SUBJECTS

(All data are given per 1.73 sq.M. of calculated surface area)

Subjects	Plasma urate, mg. %	Urine urate, mg./min.	Urate clearance, cc./min.	Glomerular filtration rate, cc./min.	$\frac{C \text{ Urate}}{G.F.R.}$
		Control subjects			
9 males	5.38	0.558	10.38	119	0.087
11 females	4.14	0.524	12.67	116	0.109
		Gout patients			
14 males	7.88	0.453	5.74	64	0.089
5 females	7.58	0.435	5.74	57	0.101

The decrease in clearance ratio appears almost entirely to account for the sex differential in plasma urate level. Differences in the actual amount of urate excreted per minute per unit surface area are slight and do not approach the magnitude of the difference in clearance ratio. Brøchner-Mortensen's data (25) show the same general tendency, with a ratio of urate to exogenous creatinine clearance of .063 for normal men and .072 for normal women.

Table 3 also suggests the importance of comparing gout patients with control subjects of the same sex when studying urate excretion. It is apparent that comparison of a gout group composed chiefly of male patients with a control group composed chiefly of women would lead to the erroneous conclusion that the ratio of urate clearance to glomerular filtration rate is significantly reduced in gout. Ultimately, this may prove to be the case, but it is not demonstrated by the data of Table 3.

DISCUSSION

The average plasma urate concentration of normal adult women is about 84 per cent of that of normal adult men. This sex differential appears

to arise at puberty, since it is not found in normal children,[2] and presumably is related to the increased rate of secretion of normal androgen which occurs at puberty. There is some rise in the plasma urate of normal females at puberty, which is to be expected since adrenal androgen production in females reaches adult levels at this time but the rise is considerably greater in normal males, presumably because of their quantitatively greater secretion of normal androgen. A similar sex differential occurs in patients with gout. The average plasma urate concentration of gouty men is higher than that of gouty women, whether gout patients are considered as a group or subdivided according to the presence or absence of subcutaneous tophi.

It is possible to give a rough quantitative analysis of factors tending to cause elevated plasma urate concentrations. One may consider the average plasma urate of normal children, 3.3 mg. per cent, to be a basal level. At puberty, normal women receive an increment averaging 0.8 mg. per cent in plasma urate, presumably reflecting adrenal androgen secretion. Men, who have considerably more androgen arising from both testes and adrenals, receive a larger increment, averaging 2.0 mg. per cent. The problem of the quantitative increase occurring in the normal woman at the menopause is under study by Smyth and his coworkers, but as yet detailed information is not available.

The increment representing the effect of normal androgen secretion (2.0 mg. per cent) is added to all urate levels in the male at puberty, whether or not the individual has inherited the gene for hyperuricemia. However, inheritance of this gene results in the addition of a further increment, averaging 2.7 mg. per cent in either sex, to the normal adult levels for that sex. It is interesting that although the magnitude of the increment controlled by the gene for hyperuricemia ultimately appears to be equal in the two sexes, the period at which the maximum urate levels are reached differs in the two sexes. One might suggest that, just as maximum urate levels are not reached in the normal woman until normal ovarian function terminates, so also the gene for hyperuricemia may not produce its maxi-

[2] Several other interesting and pertinent observations were noted in the more detailed analysis of urate metabolism in children (32). The appearance of the sex differential in plasma urate is distinctly a puberal phenomenon, since the urate levels for both sexes show no differential in the age 0–6 group or in the age 7–13 group. The average plasma urate for all individuals is only very slightly higher in the age 7–13 than in the age 0–6 group. Values for the ratio of urine urate to preformed creatinine, urine urate to total creatinine, and urate clearance to creatinine clearance were very considerably higher in children than in adults, and preliminary findings suggest that this entire pattern of differences may be diminished temporarily by the administration of potent androgens. These results will be reported in detail in future communications.

mum effect until the same period. A final differential is that which obtains between the average plasma urate of patients with pretophaceous and tophaceous gout. This averages about 3.3 mg. per cent. So far as we are aware, no one has ever studied the question of whether the severity of gout is inherited or whether the tendency to tophaceous gout is inherited, but the earlier onset of tophaceous gout in women and the more frequent family history obtained from such patients is suggestive. Most interesting of all are the occasional reports of a family history of gout in women (*see* the appended note on gout in women).

The amount of urate excreted per minute is about equal in the control men and women and in the gouty men and women. The higher plasma urate of normal men appears to be a function of the lower ratio of urate clearance to glomerular filtration rate in this sex. In turn, the smaller clearance ratio of men seems to arise from lower urate clearances and higher glomerular filtration rates in normal men. The sex differential in plasma urate, superficially at least, appears to reflect differences in the urate excretory mechanism[3] between the two sexes, rather than differences in the rate of purine turnover.

Relationship of the normal sex differential to inherited hyperuricemia. The tendency to develop hyperuricemia is inherited,[4] but the age at which this latent tendency is translated into an elevated plasma urate level seems to depend in part upon endocrine status. In either sex, actual hyperuricemia ordinarily develops at a period of life characterized by a relative increase in androgen unantagonized by estrogen. In men, both the normal sex differential and inherited hyperuricemia produce their characteristic elevations in plasma urate level at, or shortly after, puberty. At first, this might suggest that inherited gouty hyperuricemia might really be only an exaggeration of the normal sex differential, a form of inherited hyperandrogenism.

A major obstacle to interpreting inherited hyperuricemia as a type of inherited hyperandrogenism is the complete lack of signs of such a condition in the gouty. Such a disturbance might perhaps be difficult to dis-

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[3] The problem of whether the difference in excretory mechanism responsible for the sex differential is ultimately to be referred to a difference in the transport mechanism for plasma urate, or to variations in a supposed tubular reabsorption of urate, will be discussed in detail elsewhere.

[4] Most investigators now concede that the majority of all cases of gout are due to inherited factors. There remains some question as to whether severe renal impairment or increased nucleoprotein turnover (as in leukemia, lead poisoning and hemolytic anemia) can result in gout unless the inherited predisposition is also present. Since cases which raise the possibility of such "secondary" gout are distinctly unusual, they are omitted from present consideration.

cover in normal adult males, but should certainly be detectable as virilism in gouty females. However, data in the note appended indicate that virilism does not occur in gouty females. It is interesting that this lack of virilism agrees with observations of Draper, Dupertuis and Caughey (33) on the constitutional status of gouty females. They report "strong emphasis on gynic features, with minimal emphasis on the andric." A laboratory finding to be expected if gouty hyperuricemia were due to inherited hyperandrogenism would be increased 17-ketosteroid excretion in the urine of gouty patients. The actual data show very low urinary ketosteroids to be characteristic of the gouty (4). For reasons already mentioned, these low ketosteroid outputs are believed to be referable to secretion of an abnormal androgen which is not metabolized to ketosteroids.

An important further objection to considering inherited hyperuricemia as an exaggerated normal sex differential is the occurrence of a sex differential in the gouty as well as in normals. This suggests that the mechanisms controlling the sex differential and inherited hyperuricemia cannot be entirely coincident but must have some degree of independence from each other. Moreover, inherited hyperuricemia seems to consist of an elevation of plasma urate over a basic level set by the individual's sex; but the magnitude of this elevation is about equal in the two sexes and not particularly greater in gouty males, as would be expected if it depended upon the rate of androgen secretion.

Finally, it is important to note that the appearance of gouty hyperuricemia, while ordinarily conditioned by factors which seem to be dependent on androgen/estrogen ratios, may exceptionally be quite independent of such control. The data on gouty women which follow illustrate the fact that functioning ovaries may provide only relative protection against gout, since tophaceous gout in women seems generally to begin before the menopause. In the 3 cases of tophaceous gout in women for whom we know the actual date of the menopause, the onset of clinical gout preceded the menopause by 7, 20, and 21 years respectively. In 3 other women with tophaceous gout, the disease began at ages 12, 16, and 26 respectively. If one may credit the older clinical literature, some modification must also be made in the Hippocratic aphorism that gout is absent in eunuchs, since Cullen (34) found it necessary to add that gout could occur in eunuchs, if they "happen to be of a robust habit, to lead an indolent life, and to live very full." There are also exceptions to the relative freedom from gout enjoyed by children. In most cases, gout in children appears to be a complication of increased nucleoprotein turnover consequent upon leukemia or hemolytic anemia, but in some authentic cases no such factor can be found (35, 36, 37).

The lack of virilism in gouty women, the absence of clinical or labora-

tory evidence of hyperandrogenism in gouty men, the mutual independence of the sex differential and inherited hyperuricemia, and the failure of gouty hyperuricemia to exhibit an invariable dependence upon endocrine status all oppose interpretation of inherited gouty hyperuricemia as merely an exaggeration of the normal sex differential due to inherited hyperandrogenism. Since the facts nevertheless point toward some relationship between the factors which control the appearance of the sex differential and of inherited hyperuricemia, this relationship must be somewhat indirect. As data now available actually do suggest the existence of a qualitative abnormality of androgen secretion in the gouty (4), a plausible hypothesis might be that the gouty possess a second hormone with a rate of secretion parallel to that of normal androgen under most circumstances, opposed by estrogen like other androgens, and responsible for the manifest expression of inherited hyperuricemia.

The working hypothesis. Present evidence indicates that the greater frequency of abnormally elevated plasma urate levels in male relatives of the gouty than in female relatives does not mean that more men than women inherit the gene for hyperuricemia. Rather the difference in the sex incidence of abnormal urate levels depends upon two nonhereditary factors: a) the fact that male carriers develop their maximum urate levels at an earlier age than female carriers, and b) the existence of the normal sex differential in plasma urate concentration (1, 2, 3). Since a prolonged and severe hyperuricemia seems to favor the appearance of clinical gout, the sex incidence of the disease may be assumed to depend upon the male tendency to earlier and greater hyperuricemia.[5]

The simultaneous appearance of the sex differential and of inherited hyperuricemia at puberty in the male, and of inherited hyperuricemia at the menopause in women, has suggested that gouty hyperuricemia might be a sort of exaggerated sex differential due to inherited hyperandrogenism. We have already reviewed the objections which appear to make this uncomplicated view untenable and which favor a more indirect type of relationship. The finding of low urinary 17-ketosteroids in the gouty, in the presence of clinically normal androgenic status and unimpaired ability to convert administered testosterone to ketosteroids, is believed to indicate that biologic androgen activity is maintained in the gouty by an abnormal androgen which, when metabolized, is not importantly excreted as 17-ketosteroid.

The present data are consistent with the assumption that the abnormal

[5] The observation that the occurrence of clinical gout and the duration and magnitude of antecedent hyperuricemia are positively correlated should not be taken to mean that the elevated plasma urate level is necessarily the direct cause of the symptoms of clinical gout.

androgen of the gouty is one which has the particular property of producing an excessive elevation in plasma urate concentration, that it ordinarily, but not always, begins to be secreted at the same period of life when normal androgen secretion begins, and also that it is functionally opposed by estrogen. The "gouty androgen" is presumed to be of adrenocortical origin, since gout may be inherited by either men or women. Albright (38), in particular, has emphasized the fact that, even in normal individuals, the onset of adrenal androgen secretion ("adrenarche") and its cessation ("adrenopause") may not exactly coincide with the onset of function or with involutional changes in the gonad. Similar timing discrepancies in adrenal-gonad relationship in the gouty may underlie the rare cases of constitutional gout in children and of premenopausal gout in women. The suggested adrenocortical origin of the "gouty androgen" makes gout in eunuchs understandable. One may speculate that some somatic action of the "gouty androgen" is reflected in the characteristic short, stout physique of the gouty woman, and in Cullen's gouty eunuchs "of a robust habit."

Since genes appear to control individual enzymes rather than individual tissues, it seems probable that an hypothesis which localizes the inherited defect in gout in disturbed adrenocortical function must at best be incomplete. Such an hypothesis has certain advantages notwithstanding; of these not the least is its consistency with other data which suggest altered adrenocortical function to be of central importance in the mechanism of the acute gouty attack. Attacks of acute gouty arthritis may be precipitated in a majority of interval gout patients by administering several doses of adrenocorticotropic hormone (ACTH) and then withdrawing the hormone. The attack ordinarily begins from forty-eight to ninety-six hours after the hormone is withdrawn. It generally begins at that point during the temporary adrenocortical hypofunction following ACTH withdrawal when the normal subject shows evidence of abrupt "rebound" and temporary endogenous ACTH overproduction; but such evidence is lacking in the gouty, who show no rebound. The spontaneous attack of acute gouty arthritis or that produced by adrenocorticotropin-withdrawal may rapidly be terminated by the administration of adrenocorticotropin (9, 10, 19), but may return unless colchicine is also given (19). Metabolic and hematologic changes suggestive of temporary relative 11-oxysteroid deficiency are found during the prodromal periods of acute gouty arthritis of spontaneous origin, and the absence of metabolic findings of compensatory adrenocortical hyperfunction during prolonged attacks of acute gouty arthritis which resist treatment (9, 19) suggests that the gout patient may respond inadequately to relative 11-oxysteroid lack. Although these findings appear superficially to imply that a failure of tropic stimulation of the adrenal by the pituitary may be involved in the mechanism of acute gouty arthritis,

TABLE 4. PLASMA URATE CONCENTRATION IN CERTAIN ENDOCRINE AND OTHER SYNDROMES

(All patients in the table below are genetic females. The only endocrine disturbance in which altered urate metabolism has regularly been reported is active acromegaly (39–47). Most investigators have reported low urate levels and high urate outputs in active cases. Thannhauser (41) was able to reverse these changes by pituitary irradiation in one acromegalic patient. Irradiation also caused a marked drop in urine urate /urine creatinine. No change was observed in a control subject similarly irradiated.)

Number of patients	Number of samples	Plasma urate, mg. %	Plasma urea, mg. %	Urate / urea	Therapy and remarks
			Normal adult women		
20	20	4.05	27.9	0.15	All premenopausal subjects
			Acromegaly		
1	1	2.70	30.0	0.09	Early active acromegaly
1	1	4.60	43.0	0.11	Late "burned-out" acromegaly; metahypophyseal diabetes mellitus
			Cushing's syndrome*		
2	4	3.89	36.2	0.11	Preoperative.
2	3	5.93	8.5	0.70	Postoperative. Successful removal of adrenal adenoma with clinical cure in both cases.
			Nephrotic syndrome (26 pound child)*		
1	3	5.70	32.0	0.18	Before ACTH treatment
1	1	2.00	34.6	0.06	ACTH, 3×5 mg. for 4 days
1	2	4.10	24.4	0.17	First week after ACTH
1	2	3.30	18.4	0.18	Second week after ACTH. Beginning of one month clinical remission
			Pseudohermaphroditism with adrenogenital syndrome		
1	1	8.20	27.0	0.30	No therapy
—	—	—	—	—	Abdominal exploration. Unilateral oophorectomy and biopsy of second ovary. Nests of lutein cells with active mitotic figures in both ovaries
1	1	7.30	33.0	0.22	Stilbestrol, 5 mg. daily
1	1	7.70	20.0	0.38	Stilbestrol, 50 mg. daily
1	1	7.10	27.0	0.26	Stilbestrol, 65 mg. daily
1	1	6.10	27.0	0.23	Stilbestrol, 100 mg. daily
1	2	5.90	33.0	0.18	Removal of left adrenal adenoma; not cured.
			Gout in women		
14	21	7.45	39.6	0.19	See Table 5 for further details

* *See* p. 761.

a sharp drop in serum cholesterol (averaging 63 mg. per cent) which may occur early in the attack without other evidence of increased 11-oxysteroid output suggests that the adrenal is manufacturing steroids other than 11-oxysteroids at an increased rate (14).

These clinical findings which indicate 11-oxysteroid production by the adrenal to be important in the mechanism of acute gouty arthritis are paralleled by evidence that these steroids regulate urate metabolism. Table 4 presents data which suggest that altered adrenal function may be important in clinical disturbances of the plasma urate level. Numerous recent physiologic studies have indicated 11-oxysteroids to increase both the rate of urate production and urate clearance (15–19), whereas, in children, androgens may be shown to have opposing effects (32).

Our present working hypothesis assumes that, in gout, biologic androgen activity is maintained by the secretion of an abnormal adrenocortical androgen which, when metabolized, is not importantly converted to urinary 17-ketosteroid. Like other androgens, this "gouty androgen" is estrogen-antagonized. Within the limits of the flexible adrenal-gonad relationship, this androgen is secreted at the times when normal androgen is secreted. The "gouty androgen" is not necessarily responsible for attacks of acute gouty arthritis, since a gouty adrenal which secretes an abnormal androgen is reasonably suspect of responding to pituitary adrenotropic stimulation in an unusual fashion. However, the onset of clinical gout appears in most cases to be correlated with the duration and severity of antecedent hyperuricemia (1, 2). Since the abnormal androgen appears to regulate the time of appearance of inherited hyperuricemia in those genetically predisposed, its secretion would appear basic to the susceptibility to clinical gout.

CONCLUSION

1. The average plasma urate of men is somewhat higher than that of women, both in the normal and gouty groups. The differences appear to depend on differences in the excretory mechanism for urate. These differences are believed to depend upon normal androgen since they are not present before puberty.

2. Carriers of genetic hyperuricemia, an inherited biochemical lesion of gout, have been found to have normal plasma urate levels until puberty, if male, and until the menopause, if female. This suggests that androgen is

Footnote to Table 4.

* Note the resemblance between the changes in plasma urate and urea caused by discontinuing ACTH therapy in the nephrotic child and those caused by successful removal of an adrenal adenoma in the patients with Cushing's syndrome.

The purified adrenocorticotropin used was furnished through the kind cooperation of Dr. John R. Mote, Medical Director, The Armour Laboratories, Armour and Company, Chicago.

TABLE 5. CHARACTERISTICS OF GOUTY WOMEN

Reference	J.D. (47)	M.J. (47)	MCH. (47)	ANN. (47)	O.M. (46)	R.F. (46)	L.E. (47)	M.H. (47)	J.C. (47)	R.C. (47)	M.D. (48)	R.W. (49)	K.H. (28)	A.N. (28)	J.P. (30)	F.R. (30)	J.H. (50)	B.M. (35)	Average
Tophi	No	Yes	No	No	No	Yes	No	Yes	No	No	No	No	Yes	No	No	No	Yes	Yes	33%
Family history of gout	No	Yes	No	No	No	No	No	No	No	No	—	Yes	Yes	—	Yes	No	Yes	Yes	31%
Height, inches	62.0	59.0	64.5	64.0	60.0	—	61.0	64.5	66.0	64.0	61.5	—	—	—	—	—	62.4	62.4	62.7
Weight, pounds	155	133	200	210	170	—	162	173	170	175	140	185	151	—	—	—	150	180	168
Age at onset of gout	55	38	50	51	57	—	47	26	49	55	59	68	14	61	55	69	55	26	49
Age at menopause	50	45	51	50	42	—	—	57	44	46	55	—	34*	—	—	—	—	—	47
History of toxemia of pregnancy	—	—	No	No	No	—	—	No	Yes†	No	—	—	—	—	—	—	—	—	16%
Para	—	0	3	2	3	—	6	3	2	2	—	—	—	—	—	—	—	—	2.6
Gravida	—	3	5	2	3	—	6	3	2	2	—	—	—	—	—	—	—	—	3.3
Hypertension	No	No	No	Yes	No	—	—	Yes	Yes	Yes	—	—	Yes	No	—	—	Yes	Yes	58%
Serum urate, mg.%	5.9	13.7	8.4	8.0	6.7	7.4	6.7	10.2	5.0	6.8	7.0	7.0	8.6	6.5	—	—	10.6	9.7	8.1
Serum urea,‡ mg.%	48	30	33	38	37	25	34	79	35	33	—	—	41	36	—	—	52	34	39.6
Hemoglobin, Gm.	10.8	12.1	12.1	11.9	11.8	—	13.3	13.7	14.1	13.1	—	—	—	—	—	—	14.7	15.3	13.0
Erythrocytes, millions per cu.mm.	3.97	3.72	4.04	4.46	4.45	—	4.06	4.40	—	4.31	—	—	4.15	—	—	—	4.70	5.14	4.31
Hematocrit, vols. %	—	34	43	41	—	—	40	45	—	38	—	—	37	—	—	—	—	—	39.7
Color index	0.87	1.04	0.96	0.85	0.85	—	1.05	1.00	—	0.97	—	—	—	—	—	—	1.00	0.94	0.97
Mean corpuscular volume	—	0.91	1.07	0.92	—	—	0.98	1.02	—	0.88	—	—	0.89	—	—	—	—	—	0.92
Total leukocytes,§	9.1	4.9	8.4	8.9	8.0	—	10.6	5.0	—	—	—	—	6.3	—	—	—	8.0	8.8	7740
Polymorphonuclear cells	67	61	36	65	49	—	—	46	—	—	—	—	—	—	—	—	84	58	54.5%
Eosinophils	3	2	4	1	0	—	—	1	—	—	—	—	—	—	—	—	0	0	1.6%
Basophils	0	1	0	0	0	—	—	1	—	—	—	—	—	—	—	—	0	0	0.3%
Lymphocytes	25	28	56	25	46	—	—	48	—	—	—	—	—	—	—	—	16	38	38.0%
Monocytes	5	8	4	9	5	—	—	4	—	—	—	—	—	—	—	—	0	4	5.6%
Glomerular filtration rate, cc. per min. per 1.73 sq.M.	—	54	—	—	73	—	—	33	85	59	—	—	12	—	—	—	—	—	52.7
Serum total cholesterol, mg.%	323	197	196	199	—	—	—	—	—	—	—	—	—	—	—	—	—	—	229
Cholesterol esters	67%	70%	73%	69%	—	—	—	—	—	—	—	—	—	—	—	—	—	—	70%
Thymol turbidity, units	3.0	2.1	1.0	1.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.9
Thymol flocculation	0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Normal
Cephalin flocculation	0	0	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Normal
Gold flocculation	1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Normal
Serum bilirubin mg. %	0.2	0.4	—	0.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.4
Serum total protein Gm. %	8.1	7.9	7.0	7.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7.5
Howe "albumin" Gm. %	4.7	5.5	4.5	4.8	—	—	—	4.4	—	—	—	—	—	—	—	—	—	—	4.8
Howe "globulin" Gm. %	3.4	2.4	1.8	2.2	—	—	—	3.0	—	—	—	—	—	—	—	—	—	—	2.7
True albumin % T.P.	44%	49%	—	—	—	—	—	45%	—	—	—	—	—	—	—	—	—	—	46%
Alpha globulin % T.P.	14%	21%	—	—	—	—	—	14%	—	—	—	—	—	—	—	—	—	—	16%
Beta globulin % T.P.	9%	14%	—	—	—	—	—	18%	—	—	—	—	—	—	—	—	—	—	14%
Gamma globulin % T.P.	33%	16%	—	—	—	—	—	23%	—	—	—	—	—	—	—	—	—	—	24%

* Surgical menopause
† Eclampsia during first pregnancy, pre-eclampsia during second.
‡ When NPN values were given, urea nitrogen was assumed to constitute 50% of the NPN.
§ The values on patient J.H. were obtained during acute gouty arthritis, and are omitted from the averages.

TABLE 6. CONTRASTS BETWEEN TOPHACEOUS AND PRETOPHACEOUS GOUT IN WOMEN.*

(This table includes the patients summarized in Table 5 and two additional women with tophaceous gout reported by Bernstein (35).)

	Tophaceous gout	Pretophaceous gout
Number of patients	8	12
Family history of gout	63%	20%
Average age at onset of gout	27	56
Onset before menopause	100%	14%
Average height, inches	62.0	63.3
Average weight, pounds	157	174
Ideal weight, 1912 tables	136	124
Average overweight, per cent of ideal weight*	15%	23%
Average serum urate, mg. %	9.6	6.9
Average serum urea, mg. %†	43.5	36.8
Glomerular filtration rate, cc./min./1.73 sq.M.	33	72
Hypertension	67%	42%
Diabetes mellitus	0	2
Hematopoietic disease	1‡	0

* Obesity may have been minimized in the tophaceous group by the greater severity of renal impairment.

† Values are for urea, as urea. In certain of the published cases reviewed, urea values were calculated from NPN values by assuming urea-N to form 50% of the NPN.

‡ Chronic monoblastic leukemia.

important in the conversion of latent genetic hyperuricemia into an actual elevation in plasma urate.

3. Since both appearance of the normal sex differential and of inherited hyperuricemia appear favored by androgen, we have considered the possibility that inherited hyperuricemia is merely an exaggeration of the normal sex differential resulting from inherited hyperandrogenism. This suggestion appears incorrect. Virilism does not occur in gouty women. No findings suggest hyperandrogenism in gouty men. There is, in fact, evidence to indicate that gout patients secrete less, rather than more, normal androgen than do normal adults.

4. The existence of a sex differential in the gouty as well as in normals suggests that separate androgenic mechanisms regulate the sex differential and the transformation of latent inherited hyperuricemia to the manifest hyperuricemia which precedes gout. Since hyperuricemia is of restricted occurrence, the latter would appear to be an abnormal androgen with a particular ability to cause an elevated plasma urate. Since hyperuricemia

may be inherited by either sex, this abnormal androgen presumably originates in the adrenal cortex.

5. *The working hypothesis.* Our preceding report suggested that, in gout, biologic androgen activity is maintained by an abnormal androgen of adrenocortical origin which, when metabolized, does not contribute importantly to urinary 17-ketosteroids. The present study suggests that an abnormal androgen of adrenocortical origin is responsible for the appearance of an abnormally elevated plasma urate level in those genetically predisposed. This abnormal androgen appears ordinarily to be secreted under the same conditions as normal androgen and to be estrogen-opposed.

Tentatively, we have assumed the two abnormal adrenocortical androgens of the preceding paragraph to be identical. Since the onset of clinical gout appears in most cases to be correlated with the duration and severity of antecedent hyperuricemia, and since the abnormal adrenocortical androgen appears to regulate the time of appearance of hyperuricemia in those genetically predisposed, its secretion would seem to be basic to the susceptibility to clinical gout. The attack of acute gouty arthritis, on the other hand, appears more closely related to a deficient response to temporary relative 11-oxysteroid lack. The possible role of the abnormal adrenocortical androgen of gout in the production of the characteristic acute gouty arthritis remains to be clarified.

Addendum

A note on the clinical and laboratory findings in women with gout.

The accompanying paper has indicated the importance of the presence or absence of virilism in gouty women when considering an endocrine hypothesis of gout. Since there appears to have been no summary of data published on a sizable group of gouty women, this added note is intended to serve that purpose.

Table 5 summarizes individual findings in 18 gouty women. Ten patients were from our series (47) and data on the other 8 were taken from recent publications (25, 28, 30, 48, 49, 50). Virilism was not reported by any investigator, and did not occur in our group. There does appear to be a characteristic physique, consisting of marked obesity and relatively short stature. In addition to the available height and weight values in Table 5, several other reports mention this body configuration without actually giving heights or weights.

Table 6 clearly shows the marked differences between tophaceous and pretophaceous gout in women. Tophaceous gout in women begins before the menopause. It is associated with considerably more impairment of renal function and with higher plasma urate levels than pretophaceous gout. A family history of gout was obtained in more than half of the

patients with tophaceous gout, but in less than a quarter of those with pretophaceous gout. This suggests that the earlier onset and greater severity of tophaceous gout in women is not due to casual factors, but is in some way genetically determined.

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THE CONSTANCY OF THE SERUM PRECIPITABLE OR PROTEIN-BOUND IODINE IN HEALTHY ADULTS

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IT has been demonstrated that the level of serum precipitable or protein-bound iodine varies with thyroid activity (1–6). As a result of these studies it is evident that in healthy subjects without disorders of this gland the organic iodine fraction in plasma is usually somewhere between 4 and 8 gamma per cent. It is not known, however, whether or not this serum precipitable or protein-bound iodine value changes from time to time within this range in any euthyroid individual. This possibility cannot be excluded in view of the knowledge that not only the level of thyroid activity but also extra-thyroidal factors may alter the value of the organic iodine fraction in serum (7, 8). The studies herein reported establish, however, the relative constancy of the serum precipitable iodine in healthy young adults, male and female, during a period of several weeks.

MATERIALS AND METHODS

Levels of serum precipitable or protein-bound iodine were measured at about weekly intervals in 4 women and 2 men, 21 to 32 years of age, during periods of one and a half to two months. In all of the female subjects the onset and duration of menses were recorded. In addition, 2 women kept a daily chart of the body temperature (oral) determined during a five-minute period before arising.

Venous blood was withdrawn in the morning, one to three hours following a light, or in the case of one of the male subjects (R.G.), a complete breakfast. The precipitable iodine concentration was measured by the method of Barker (9) with slight modifications (10). Recoveries of potassium iodide, diiodotyrosine, and thyroxine were 95 per cent and better in concentrations corresponding to the serum values obtained in this study.[1] In 2 studies, J.M. and J.G., the sera were analyzed shortly following the

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[1] We are indebted to Dr. H. Sidney Newcomer of E. R. Squibb and Sons for the thyroxine used in these studies.

venipuncture. In the other 4 the sera were precipitated and washed in the usual manner, stored at −10°C. until the completion of the experiment, and then analyzed as a group. Results of analyses in duplicate or triplicate agreed, on the average, within less than 0.3 gamma per cent. The findings are presented in Figures 1 through 3.

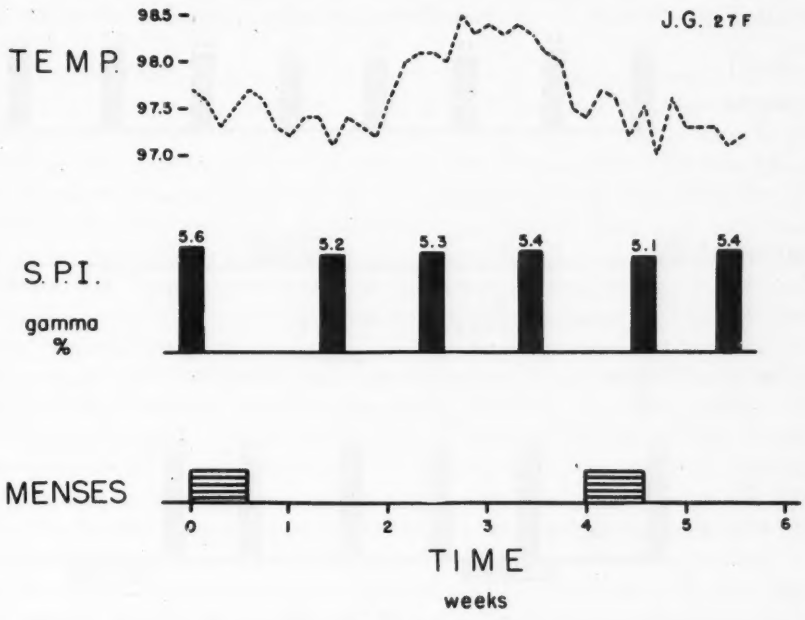

FIGURE 1

DISCUSSION

All of the individuals studied appear to have had euthyroid levels of serum precipitable iodine, falling within the 4 to 8 gamma per cent range described for subjects without thyroid disorder. The maximum variations in the serum precipitable iodine in any one of the 4 women were only 0.5 to 0.8 gamma per cent during the 6 to 8 analyses which were made. The smallest variation. 0.3 gamma per cent, was observed in R.G., one of the two male subjects (Fig. 3). This particular subject, it will be recalled, was the only one with a regular intake of a complete breakfast one to two hours prior to withdrawal of the blood sample. The largest change in the serum precipitable iodine, 1.1 gamma per cent, occurred in the second male subject (J.P., Fig. 3). It is clear from these findings that the serum precipitable or protein-bound iodine concentration varies but slightly from week to

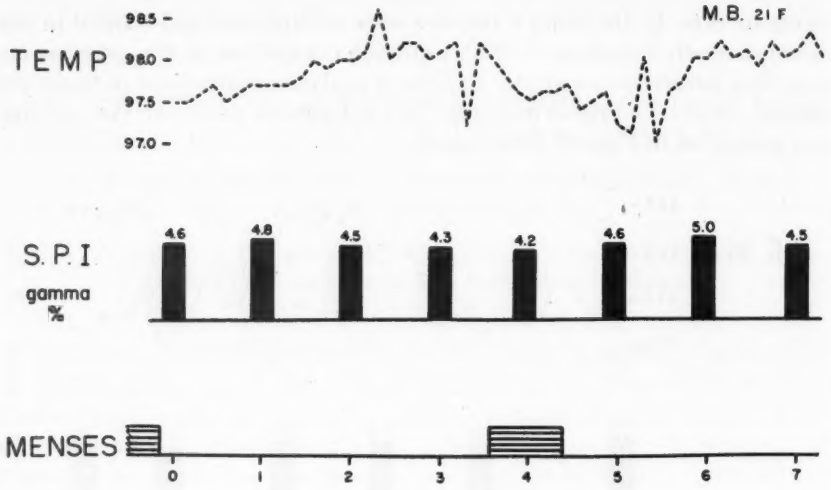

FIGURE 2

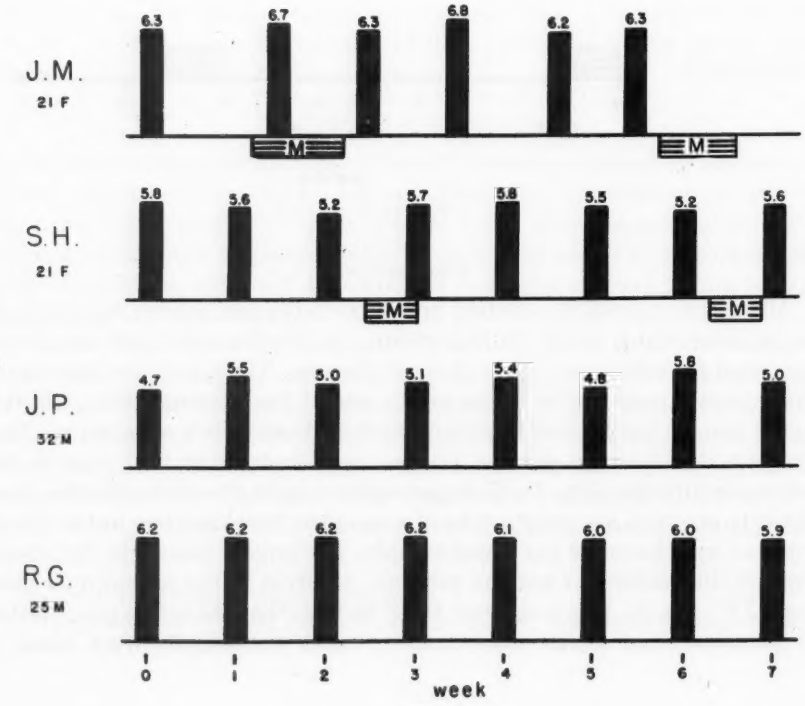

FIGURE 3

week. In evaluating the relative constancy of the serum precipitable iodine in these 6 subjects it should be kept in mind that they were all in good health. Their dietary habits remained fairly constant and presumably their levels of serum protein were unaltered. Neither did they receive inorganic iodide in any form other than that present in their usual diet, nor were they given any organic iodine compounds. Significant alterations in any of these factors are known to affect the levels of serum precipitable iodine (7, 8, 10).

The possibility cannot be excluded that some variations were occurring in these subjects in either the release of hormone from the thyroid gland or in its utilization or degradation. This is particularly true in view of the finding that in all but one of the subjects, the difference between the highest and lowest values exceeded the 0.3 gamma per cent analytical error. Inspection of the data presented graphically suggests that there may have been a cyclic variation in the level of serum precipitable iodine in some of the studies. To determine whether or not these variations are statistically significant the data were subjected to the following analysis: the "t" test was applied, using one or more sets of low and high mean values, and the dispersion of the individual values contributing to the mean.[2] The results of the "t" test together with the degrees of freedom, "n," were used to calculate a probability value, "p." In a total of 4 instances in 3 of the female subjects (M.B., J.M., and S.H.) "p" is less than 0.05, *i.e.* the observed differences could have occurred by chance only less than 5 times in a 100, and hence they may possibly be significant. No statistical significance can be assigned to the changes in the other female subject (Figure 1) since "p" is less than 0.30. The correlation of these slight but statistically significant changes in the serum preceipitable iodine values with physiologic events is admittedly difficult and perhaps impossible. It is of interest, however, to speculate upon one possibility. Studies of the serum precipitable iodine in women indicate that the concentration of this fraction usually increases with pregnancy (11, 12, 13). This appears to be an early physiologic response, antedating the rise in the basal metabolic rate characteristic of the second and third trimesters (14, 15). Certain of the endocrine changes which characterize pregnancy, such as the decrease in follicular secretion and the rise in corpus luteum activity, also occur to some degree during the menstrual cycle. They may conceivably exert an effect on the serum precipitable iodine. Study of Figures 1, 2, and 3 reveals that in the course of periodic measurements of the serum precipitable iodine in the 4 female subjects probably all aspects of the menstrual cycle were covered. The 2 subjects who determined their daily basal temperature, J.G. and M.B.,

[2] Mr. Carrol S. Weil of the Mellon Institute kindly carried out the statistical analyses of the data.

showed the characteristic rise during the postovulatory phase of the cycle (16, 17). This terminated with a drop in the basal temperature just before or with the onset of menses. It seems reasonable to assign some, and perhaps all, of the sporadic dips or peaks in body temperature during the trends, to extrinsic factors unrelated to the menstrual cycle itself. Analyses of the precipitable or protein-bound iodine were run on serum obtained on one or more occasions within a few hours of the onset of menses, just after the onset, during the period itself, and shortly following its termination. Also, it is probable that the ovulatory phase was covered in a similar manner although conclusive evidence for this is, of course, lacking. Study of Figures 2 and 3 indicates, as might be expected, that it is not possible to detect during the menstrual cycle any definite elevation in serum precipitable or protein-bound iodine comparable to that observed early in pregnancy. However, if any trend is present, in subjects M.B., J.M. and S.H., it is toward a mid-point rise followed by a decrease during the postovulatory phase. It may be, therefore, that with pregnancy this slight rise is accentuated and maintained, and that in its absence the serum precipitable iodine declines to a lower level. The occurrence of equally great, if not greater fluctuations in the protein-bound iodine in one of the male subjects, J.P., might be viewed as evidence against such a theory. However, it by no means excludes the possibilities which have been presented, since thyroid function and levels of circulating protein-bound iodine do vary with factors other than pregnancy.

SUMMARY

1. The concentration of serum precipitable or protein-bound iodine appears to be relatively constant over a period of weeks in healthy young adults.

2. The hypothesis is cautiously advanced that the elevation in serum precipitable iodine which usually occurs in pregnancy may represent an accentuation and prolongation of a rise observed between menstrual periods.

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A MASCULINIZING TUMOR OF THE OVARY IN A POSTMENOPAUSAL WOMAN

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THERE has been, and there still is, considerable confusion in the literature concerning certain masculinizing tumors of the ovary, especially the so-called adrenal rest tumor, or lipoid cell virilizing tumor, or masculinovoblastoma, or hypernephroma of the ovary. The multiplicity of names, each including some characteristic of the tumor, bears testimony to the need for clarification.

In 1908 Bovin (1) reported the first case of hypernephroma or adrenal rest tumor of the ovary. Stadiem (2) reported 19 cases of hypernephroma of the ovary in 1937. In a critical review of these cases in 1939, Rottino and McGrath (3) could not classify any of them as virilizing lipoid cell tumors of the ovary, and limited the total number reported in the literature to 7 cases. Kepler (4) in 1944 reviewed 6 additional cases and reported the fourteenth. Greene (5) in 1944 reported a case of adrenal rest tumor of the ovary, and Burket and Abell (6) later in the same year reported still another case. At the present time Curtis (7) declares that only 16 well authenticated and undisputed cases can be found in the literature. The case reported here can be added to the growing list. It is hoped that the information furnished by this report may add to the clinical and pathologic data so that eventually diagnostic criteria will catalogue this clinical entity more specifically.

CASE REPORT

R. R., XL 106160, a white female, aged 65, was admitted to the Indiana University Medical Center on January 17, 1947, complaining of weakness, and of "turning into a man." Four years prior to admission she began noticing hair on her face, body and extremities. She required shaving daily. At the same time her voice began to "crack" and became deeper than usual. Her skin became darker, and she developed pigmented hyperkeratotic spots on the face and body. The weakness complained of was in spells rather than continuously and on a few occasions resulted in a fall. It was relieved in part by taking something sweet. There had been no weight change, the patient always having been obese. She had a normal menopause at the age of 53.

In the past history, a diagnosis of diabetes was made at the age of 54, and again at 59, when she required up to 40 units of insulin daily. Later she discontinued both insulin and diet as she began to develop the weak spells of her present illness. For many years there had been a known hypertension as high as 240 systolic, with an average of 180 systolic.

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The past history is almost a surgical calendar, with cholecystotomy at 36 years of age, cholecystectomy and appendectomy at 45. At 53 she had an exploratory laparotomy for fibroids of the uterus, but they were not excised. Instead, she was told, a section of bowel was removed. At 54–55 years a right iridectomy and cataract removal was done. Then at 59 years she underwent a left iridectomy and removal of a cataract. Mild diabetes, uterine fibroids and hypertension were confirmed during this latter hospitalization.

Physical examination revealed a healthy-appearing elderly white female, definitely obese and with heavy-lensed spectacles. Her temperature was normal, weight 217

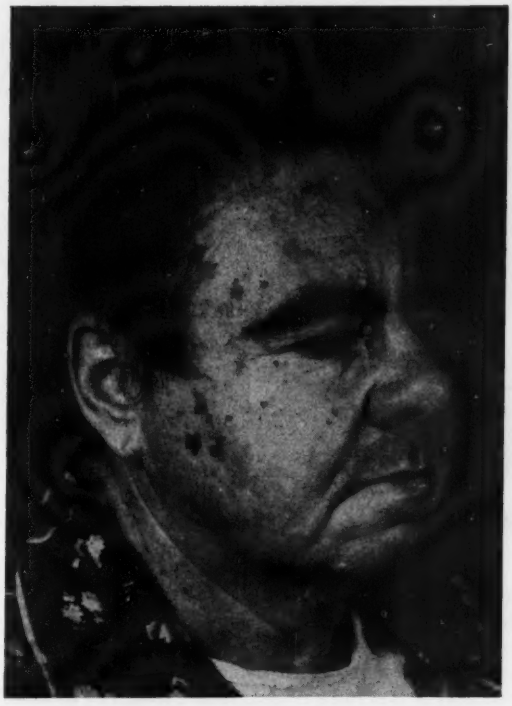

FIG. 1. Showing recently shaved beard and receding hairline.

pounds, pulse 76, and blood pressure 196/90. The scalp hair was noticeably thin and fine, with recession of the temporal hair line. There was coarse hair over the face, trunk, and arms with male distribution of the pubic hair (Figs. 1 and 2A). The distribution of fat was normal with the exception of a buffalo-type shoulder and neck contour. The skin was dry, coarse and brown. There were numerous pigmented elevated lesions, and raised waxy irregular lesions on the face, neck, upper trunk and arms. Eye, ear, nose, and throat examination revealed nothing abnormal except for bilateral iridectomies. The thyroid gland was not enlarged. The breasts were pendulous, and the abdomen showed an excess of adipose tissue. The chest was clear. The heart was not enlarged. There was a soft, short systolic murmur at the apex, not transmitted. The obese, pendulous abdomen

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had numerous old surgical scars, but no striae. The viscera were not palpable and no masses were palpated in the renal areas. Pelvic examination revealed a clitoris 2½ centimeters in length and 1 centimeter in diameter (Fig. 2B). There was a nulliparous vagina and a small soft cervix. The uterus showed multiple uterine fibroids on bimanual examination. Adnexal areas could not be adequately examined because of the obesity and the fibroids. The extremities were normal.

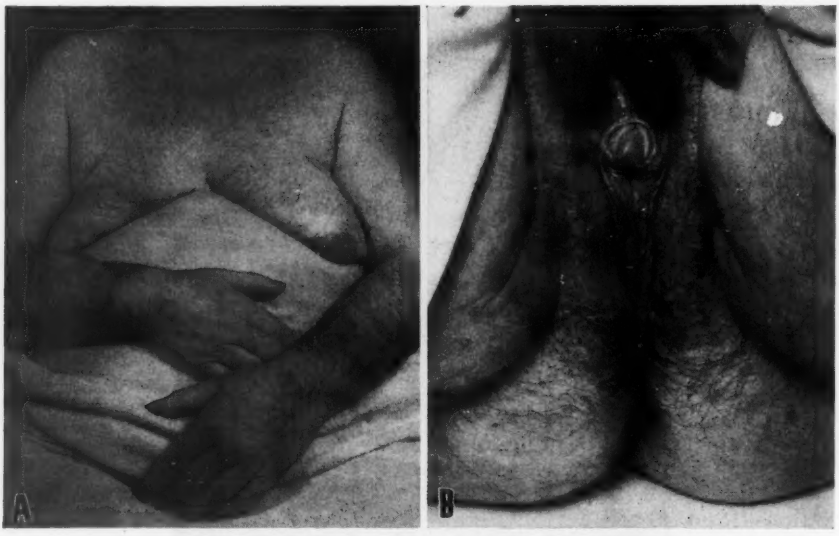
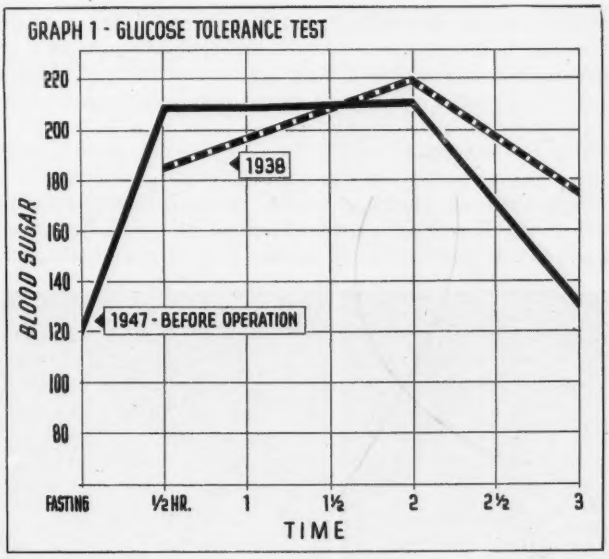


FIG. 2. A. Showing hirsutism of chest and arms. B. Showing hypertrophied clitoris 2½ cm. in length and 1 cm. in diameter.

Laboratory examinations. Roentgenograms revealed a normal chest and skull. Examination of the renal areas by spot films, as well as intravenous pyelograms, showed nothing abnormal. The urine was normal and the Wassermann and Kahn tests were negative. The blood count on admission showed a hemoglobin of 16 grams, red blood cells 4.8 million, white blood cells 10,200 and a normal differential. The total nonprotein nitrogen was 28 mg. per cent, and the phenolsulphonphthalein urinary excretion was 50 per cent in one-half hour. Total serum protein was 6.9 Gm. per cent with albumen 5.3 grams and globulin 1.6 grams. The serum chloride level was 618 mg. per cent, cholesterol 185 mg. per cent and the cholesterol esters 59 mg. per cent. The Friedman test was negative. A twenty-four hour urine specimen contained 146.5 mEq. of sodium per liter and 32 mEq. of potassium per liter. The urinary excretion of 17-ketosteroids in twenty-four hours was 43 mg. Oral glucose tolerance and glucose-insulin tolerance tests showed a mild diabetic tendency and insulin resistance (Fig. 3). Basal metabolic rates on successive days were plus 8, plus 5, and plus 17 per cent. The histamine phosphate test showed no significant change in the blood pressure after the intravenous injection of 0.05 mg. histamine phosphate. The cold pressor test was normal.

Surgery. On February 13, 1947, a laparotomy was performed. The adrenal areas were palpated through a left upper rectus incision and it was thought no tumor was present.


GRAPH 1 - GLUCOSE TOLERANCE TEST
BLOOD SUGAR
220
200
180
160
140
120
100
80
1938
1947 - BEFORE OPERATION
FASTING
½ HR.
1
1½
2
2½
3
TIME

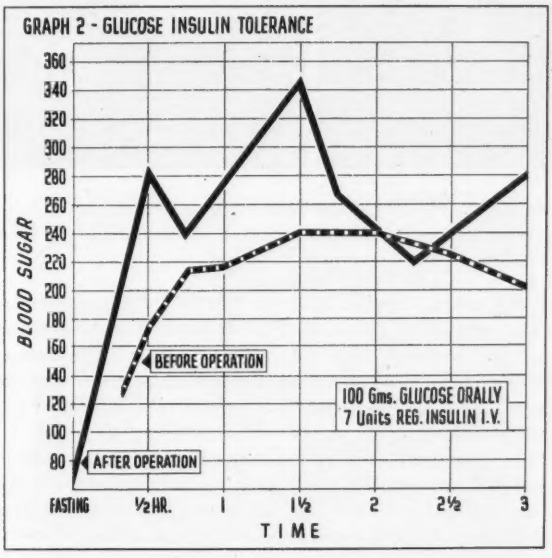

GRAPH 2 - GLUCOSE INSULIN TOLERANCE
BLOOD SUGAR
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
BEFORE OPERATION
AFTER OPERATION
100 Gms. GLUCOSE ORALLY
7 Units REG. INSULIN I.V.
FASTING
½ HR.
1
1½
2
2½
3
TIME

FIGURE 3

A huge fibroid uterus was found and the right ovary was replaced by a tumor mass about 3 inches in diameter. It had not invaded any of the surrounding tissues. The tumor was removed with some difficulty through a right McBurney incision. The patient's condition throughout the operation was good; however, the blood pressure dropped to 140 systolic during the last half hour.

Postoperatively the patient received a total of 365 mg. adrenal cortical extract (Percortin) over a six-day period, and 9 grams of sodium chloride daily in excess of her food intake for the first week. The blood pressure gradually increased during this period to preoperative levels. The temperature was normal at the fifth postoperative day. Laboratory examinations postoperatively and before discharge revealed a normal blood

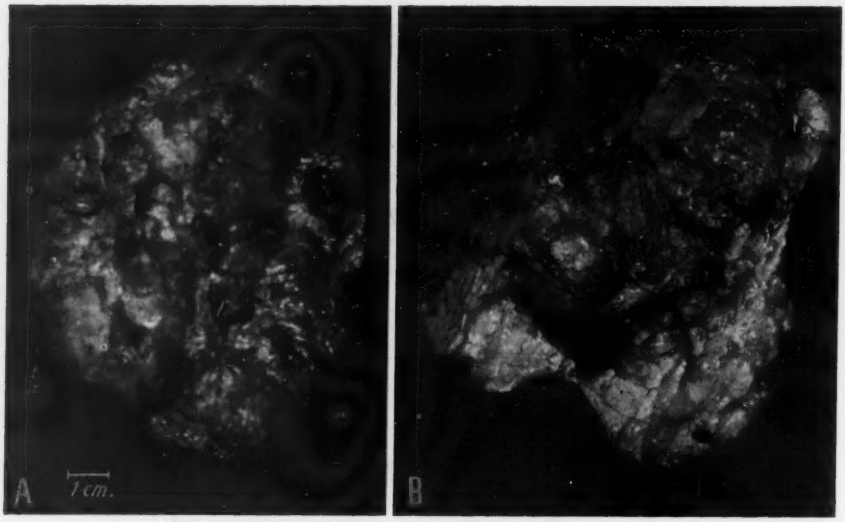


FIG. 4. A. Tumor of ovary. Gross specimen intact showing marked lobulation. B. Cut section showing solid character of the tumor and lobulation produced by pseudocapsules.

count and urine. The serum chloride level was 680 to 711 mg. per cent, serum sodium 127 mEq./liter and serum potassium 0.43 mEq./liter. The 17-ketosteroids were reported as 1.7 mg. in the twenty-four hour urine specimen. There was no significant change in the glucose-insulin tolerance test, the patient still showing an insulin resistance. At the time of discharge the patient was told to take stilbestrol 1 mg. daily three weeks out of every month.

Pathologically the specimen consisted of a tumor measuring 10 cm. in length, 8 cm. in greatest width, and 4.5 cm. in thickness (Fig. 4). Its external surface showed a capsule which was thin and did not appear to be intact over the entire specimen. The tumor mass was lobulated, and was homogeneous yellow in color except where the capsule was stained by blood. The lobular arrangement of the tumor was seen on cut section with the color again a bright yellow and soft in consistency. At one extremity of the tumor was an area softer than the remainder and somewhat red in color. This was thought to represent necrosis. The tumor weighed 130 grams.

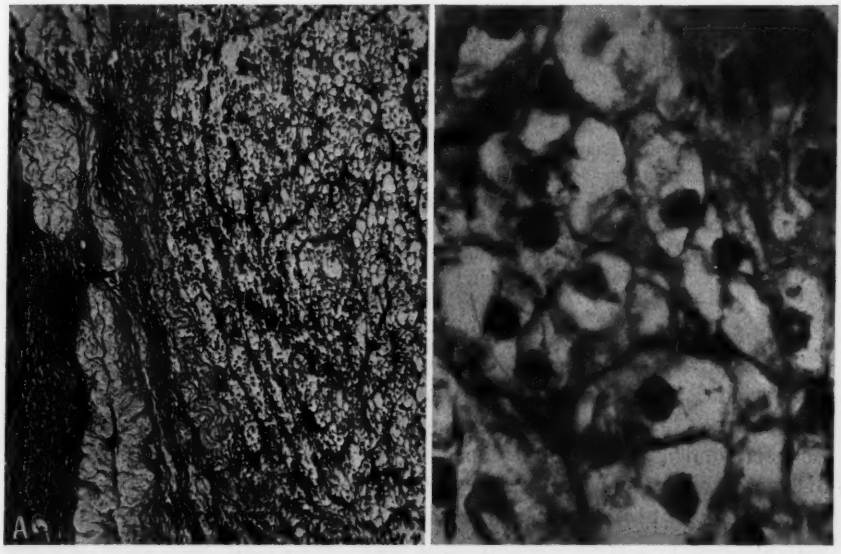


FIG. 5. A. Low power photomicrograph showing tumor on the right and ovarian tissue on the left. B. High power photomicrograph of tumor showing similarity to adrenal cortical cells.

Microscopic sections of the tumor revealed a tissue capsule surrounding tumor cells (Fig. 5). The capsule appeared normal for ovarian tissue. There were corpora albicantia present. The major portion of the tumor consisted of large polyhedral cells suggestive of lipoid-containing cells or adrenal cortical cells with deeply staining granular nuclei. These

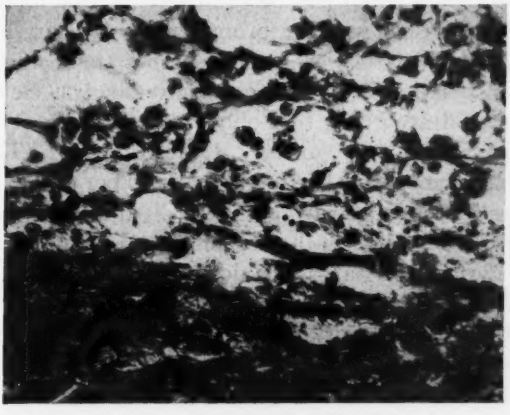


FIG. 6. Sudan III stain showing lipoid content of cells.

cells appeared to be arranged in strands or columns with a fine reticular stroma between the columns. The fine reticular stroma separating the surrounding cells appeared to be rather vascular with small papillary channels filled with red blood cells. The individual cells, in themselves, had a fine reticulum within the cytoplasm. With Sudan III stain, the cells were packed with fat (Fig. 6), and chemical analysis of the tissue showed it to be composed of approximately 85 per cent fat (8).

Postscript: A one year follow-up on this patient has shown a loss of most of the hair of body and extremities. The patient shaves three times weekly. Weight and blood pressure have remained as previously. The clitoris is grossly smaller and softer. The masculine characteristics have receded but have not disappeared. The patient had homologous serum jaundice 3½ months after leaving the hospital.

DISCUSSION

The symptoms and signs associated with this tumor are beginning to be fairly well defined. However, the endocrine glands are so interrelated that the influence of the various hormones on one another has led to difficulty. As hormonal assays become more widely used it would seem that more distinct evaluation of clinical syndromes will be possible. In this type of tumor the symptoms as a rule are fairly well defined and consist of first defeminizing and later masculinizing signs and symptoms. This case was in a postmenopausal female who developed virilism and hirsutism. If Maxwell's (9) case is excluded as a typical masculinovoblastoma, this case is the only one occurring following the menopause. Voice changes were present but not to a marked degree. With Iverson's (10) table of clinical and morphologic features as a criterion, this patient fits in with those previously reported. The duration of the disease was four years; there was no weight loss or gain in the already obese individual; there was no pain referable to the tumor mass; and there was no abdominal swelling or palpable mass. Libido could not be evaluated in this unmarried woman. As mentioned, hirsutism was the prominent symptom. The clitoris was hypertrophied. The breasts were pendulous but there was no remarkable atrophy of breast tissue. The 17-ketosteroid value was elevated to 43 mg. This was estimated according to Robbie and Gibson's (11) rapid clinical method for extracting total urinary 17-ketosteroids. (The twenty-four hour specimen of urine, after hydrolysis with 10 per cent HCl, is extracted for ten minutes by refluxing with boiling CCl_4 and the hormone content is determined by the alkaline m-dinitrobenzene technique.) No estrogen or gonadotropic hormonal assays were done, as the medical institution was not equipped to do these laboratory determinations. Hypertension had existed a number of years prior to the onset of hirsutism. Eleven years before this hospitalization the patient was told she was a diabetic, and in 1940 she was given insulin for a period of months. The basal metabolic rate was within normal limits. These clinical and laboratory data fit in with the pathologic picture of a virilizing lipoid-cell tumor of the ovary.

At present we must rely mostly on evidence in the field of clinical and pathologic diagnosis for the classification of these tumors. The adrenal rest tumors of the ovary are very much alike in their microscopic picture but may vary in details enough to be confused with arrhenoblastoma(12). Our tissue has been submitted to the Gynecological Tumor Registry and has been classified by them as an adrenal rest tumor of the ovary (synonyms being masculinovoblastoma, hypernephroma of the ovary, adrenal cortical cell tumor of the ovary, and virilizing lipoid cell tumor of the ovary).

SUMMARY

1. The clinical picture of a masculinizing syndrome in a postmenopausal woman, age 65, is presented and discussed.

2 A tumor mass which replaced the right ovary was removed.

3. Pathologically the tumor followed the picture described for an adrenal cortical cell tumor of the ovary.

4. This case is reported in an attempt to increase the knowledge of the clinical entity produced by a masculinizing tumor of the ovary, or masculinovoblastoma.

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PHEOCHROMOCYTOMA WITH HYPOTHALAMIC MANIFESTATIONS AND EXCESSIVE HYPERMETABOLISM

A CASE REPORT

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THE apparently hypothalamic origin of certain functional disturbances occurring in cases of pheochromocytoma has only rarely been emphasized. A case is reported here in which such manifestations were present as well as an extraordinarily high elevation of the basal metabolic rate and a sustained arterial hypertension.

CASE REPORT

M. T., a 28-year-old unmarried white female mill worker, was examined in Burlington, Vermont, on October 22, 1942. *Family history*: Parents, 5 sisters and 1 brother alive and well; 2 brothers died in early childhood of unknown causes. *Past history*: Scarlet fever, measles and mumps during childhood; otherwise always well. Menstruation began at age 14 and was always regular. She did not smoke tobacco nor drink alcoholic beverages.

Present illness:—During the year previous to examination she felt easily fatigued and lost weight (from 148 to 138 pounds) despite an excellent appetite and normal temperature. There was some palpitation and occasional shortness of breath, both on exercise and at rest; no dizziness; occasional moderate headache; and a slight degree of nervousness and irritability. A dusky red and bluish discoloration of her hands and legs developed during the year without being accompanied by any abnormal sensations. Once spontaneous blanching of one finger occurred, lasting twenty minutes. Her chief complaint was a continuous, intense perspiration from the waist up by day and night. Medication with Lugol's solution for five months had no effect.

Physical examination:—(1942). The patient was 5 ft. 6 in. tall, had a normal figure and healthy appearance. She was calm and cooperative. There was no conspicuous nervousness or motor restlessness, no exophthalmos or stare. The pupillary reflexes were normal. Some acne pustules were seen on the forehead, chin and back. The skin was very moist, especially so on the trunk and hands. There was an intensely dark, bluish-red, mottled discoloration of the lower legs and, to a lesser extent, of the hands. The discoloration faded markedly on elevation of the limbs and disappeared for a moment on local pressure. There was no edema. The deep reflexes were sluggish. There were no abnormal reflexes and no tremors. There was no enlargement of the thyroid. No glands were palpable.

The lungs appeared normal to percussion and auscultation. There was a constant

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tachycardia of about 120 beats per minute, a short systolic murmur over the apex and accentuation of the second aortic sound. The blood pressure was 220/130.

The abdomen was normal. The hymen was intact. The uterus was small and retroverted.

On the right eye ground, blurring and hemorrhages were seen in the lower temporal region of the disc margin and along the vessels extending into the fundus; the left eye showed papilledema and distended and tortuous veins. The ears were normal.

Initial laboratory and X-ray findings:—The electrocardiogram showed the following details; sinus rhythm, left axis deviation, PR 0.16 seconds, QRS 0.05 seconds, RT very slightly depressed in leads 1 and 2, T abnormally low in leads 1 and 2. On roentgen examination the heart appeared normal in size, shape and position; there was no indication of a substernal goiter. The pyelogram showed normal position of the kidneys and no abnormal shadows. A skull plate showed a normal sella turcica and no signs of increased intracranial pressure.

Examination of the blood showed a color index of 0.9, a count of 4,600,000 red cells, 12,100 white cells and a normal differential. The sedimentation rate was 17 mm. per hour. The Wassermann and Eagle tests were negative.

The basal metabolic rate was plus 69 per cent (one month earlier it had been plus 97 per cent).

Clinical course:—The patient felt subjectively well until the night of February 1, 1943, when she had a sudden attack of cough, dyspnea, choking, expectoration of frothy sputum, and cyanosis, which lasted for two hours and from which she recovered rapidly without a recurrence.

On June 5, 1943, she suddenly developed high fever (about 104° F.) which lasted for three days. She was restless, the heart rate was 140, but examination did not reveal any other abnormalities. Subsequently she felt exhausted for a month and she suffered from intense thirst which compelled her to drink 4 to 5 liters of water daily and to urinate six to eight times every night. At the same time, there was an irresistible craving for carbohydrates. She used to get up two or three times every night to eat a dishful of cereal each time. Menstruation ceased abruptly. Her weight dropped from 125 to 116 pounds. Within five months the polydipsia and polyphagia disappeared, menstruation reappeared, her weight returned to its former level and she resumed her work without further difficulties.

From November 1943 to January 1944, she received a daily dose of 0.6 Gm. of thiouracil. During this treatment the pulse rate decreased slightly (to about 110 per minute), but the basal metabolic rate and the blood pressure remained practically unchanged at a high level. The further clinical course from 1942 until her admission to the Massachusetts Memorial Hospital in Boston in 1947 was uneventful, except for a miscarriage three months after her marriage in 1946.

During these five years she was frequently examined, however, and the following findings were obtained:

Blood Pressure: fluctuated between 146 and 220 systolic and 106 and 130 diastolic, remaining mostly at the upper levels. Physical exercise was accompanied by a fall of the systolic and diastolic pressures, *e.g.*, from 146/102 to 132/0. The pulse rate rose simultaneously from 120 to 160 per minute. Subcutaneous injections of 0.1, 0.2 and 0.4 mg. of adrenalin did not affect the blood pressure level; intravenous injection of 0.2 mg. caused a sudden rise from 170/100 to 260/140, followed by a return to normal within four minutes. Intramuscular injection of 0.05 mg. of histamine was followed by an elev-

tion of the systolic pressure of only 20 mm., and of the diastolic pressure of 10 mm. A benzodioxane test was not done, as this test had not yet been published before surgery was undertaken. The cold pressor test had no appreciable effect on the systolic and diastolic pressures. Sedation caused a maximum fall of the blood pressure to 150/90 mm. Postural fall of the blood pressure was repeatedly observed (*e.g.*, from 190/120 to 146/106) and was accompanied by cardiac acceleration (*e.g.*, from 108 to 140 beats per minute.)

Most striking was the blood pressure-depressing effect of slow hyperventilation in the recumbent position (Fig. 1). Both systolic and diastolic pressures fell to entirely normal levels within 15 minutes, while the heart rate remained unchanged.

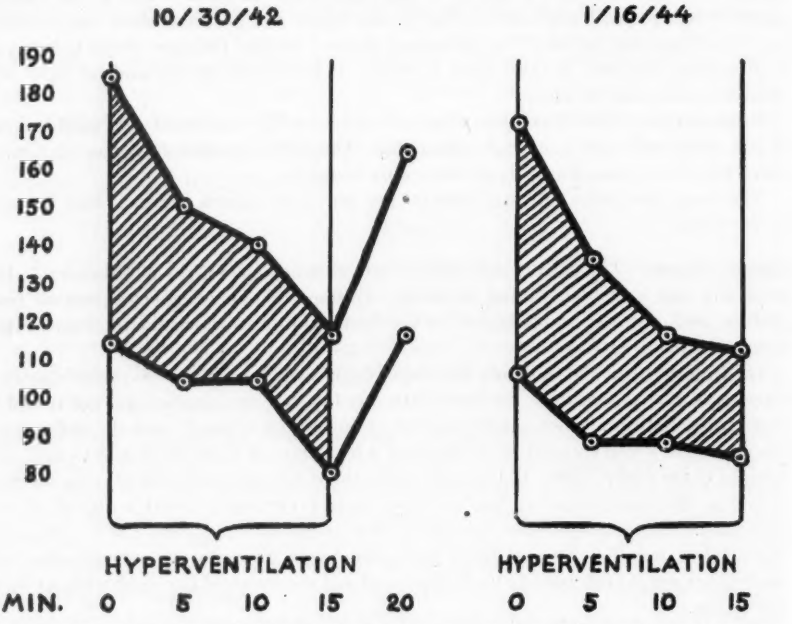

FIG. 1. Fall of systolic and diastolic blood pressure during fifteen minutes of hyperventilation, followed by the usual return toward the previous level after cessation of hyperventilation in the first experiment. The second experiment was interrupted.

Basal metabolic rate determinations were made twelve times between 1942 and 1945. During this period the B.M.R. varied between plus 69 per cent and plus 142 per cent, remaining mostly above plus 100 per cent. These readings were not affected by plugging the ears in order to eliminate a possible error through escape of oxygen. In 1947 the B.M.R. was considerably lower: plus 22, plus 33 and plus 36 per cent.

Urine specific gravity was 1014 to 1032. Albumin was 0 to 1.5 per cent (usually 0), sugar was 0 to 1.7 per cent (usually 0) and acetone was 0 to 3 plus (positive after twenty-four hours of a carbohydrate-free diet but also without such provocation). P.S.P. excretion in two hours was 30 per cent in 1944 and 51 per cent in 1947. Creatinine excretion in twenty-four hours was 1.9 Gm. (1944). In a 24-hour specimen the 17-ketosteroids were 3.5 mg. (1944, courtesy of Dr. H. Friedgood, Harvard Medical School).

Blood chemistry: The blood sugar level (fasting) was 95 to 118 mg. per cent. A glucose tolerance curve was normal in 1944 and showed mild diabetes in 1947. N.P.N. was 27 to 40 mg. per cent, creatinine 2 to 6 mg. per cent, cholesterol 233 to 320 mg. per cent, calcium 9.9 to 12.1 mg. per cent, phosphorus 3 to 6 mg. per cent, phosphatase 2.1 units, total iodine 11.3 micrograms per cent (courtesy Mr. H. J. Perkin, Lahey Clinic, Boston), serum proteins 7.2 Gm. per cent, chlorides 104 milliequivalents per liter and alkali reserve 28.6 milliequivalents. Prothrombine time was normal. Adreno-sympathetic catechol compounds in the blood were within normal limits on two occasions (colorimetrically assayed). On the isolated frog heart the serum caused a moderate increase of the amplitude.

Electrocardiogram: Unchanged from 1942 to 1945. In 1947: T1 upright, T2 diphasic (− +), T3 inverted and T upright in CF4, 5. Normal axis position.

Heart size: (X-ray) 12.5 cm. in 1942, 13.1 cm. in 1947. The inner diameter of the chest was 27.5 cm.

Electroencephalogram: (Dr. Toman) Normal with moderately strong alpha rhythm.

Eye grounds: Somewhat improved after 1944. In 1947: grade 2 hypertensive and grade 1 sclerotic changes.

Surgery: After repeated and prolonged persuasion, the patient finally entered the Massachusetts Memorial Hospital in Boston in 1947 and on April 24 of that year the right sympathetic trunk was removed from D-10 to L-1 inclusive and the great splanchnic from just above the celiac ganglion upward to the level of D-10. On the left side a tumor of the adrenal gland was found and resected.

At the time of the removal of the tumor the blood pressure dropped precipitously and this critical condition remained the same following the operation. It necessitated the infusion of a total of 54 mg. of adrenalin within seventy-two hours in addition to substantial amounts of blood and plasma. On the third postoperative day the blood pressure became stabilized. For six days she remained mildly disoriented but on the fifteenth postoperative day she was discharged in good condition. At that time the blood pressure ranged from 110/72 to 135/100 and the pulse rate from 78 to 100.

When she was seen one month later in Burlington, she felt perfectly well. The discoloration of legs and hands and the excessive perspiration had disappeared. The blood pressure was 104 to 116 systolic, 78 to 80 diastolic and the pulse rate was 81 to 144 (the latter only in the standing position). The electrocardiogram was normal. The urine was normal. P.S.P. excretion was now 70 per cent in two hours. The B.M.R. was plus 2 per cent. According to recent reports she is still in excellent health. She gave birth to a 9 lb. 6 oz. child on Jan. 27, 1949.

Pathology of the tumor:—(Dr. J. Diamond) The adrenal tumor measured 4.3 cm. in length, 3.1 cm. in width and 2.5 cm. in thickness. It weighed 20.7 grams with a minute remnant of uninvolved adrenal tissue at either pole. It showed thin yellow patches of cortical tissue beneath the capsule and small areas of hemorrhage. The medulla was reddish-gray and soft. In the mid-portion there was a small cyst which contained thin, red fluid.

Microscopically the tumor was identified as a pheochromocytoma, consisting of adrenal medullary cells arranged in compact cylindrical cords which were bounded by fibrocollagen. The cells varied from a small round form to a moderate sized fusiform shape, interspersed with many extremely large cells whose boundaries were indistinct. The cytoplasm was eosinophilic in all types of cells, finely granular with a fine reticulation and occasional vacuolization. The larger cells contained very large nuclei with

prominent nuclear membranes; in the smaller cells the nuclei were round or ovoid and vesicular. No mitoses were seen.

Bio-assay of the tumor was carried out by Dr. O. Krayer of the Harvard Medical School with tests on blood pressure and isolated gut. It revealed that tumor extracts exerted activities corresponding to an epinephrine equivalent of 0.3 mg. per gram of fresh tissue.

Kidney tissue removed during the operation showed the following histologic details: "occasional arterioles and pre-arterioles present slight intimal sclerosis and/or hyaline deposition, and a few arteries show slight to moderate intimal sclerosis. There are three hyalinized glomeruli. The other glomeruli are normal or show only slight hyaline thickening of capillary basement membranes." Diagnosis: nephrosclerosis, grade 1.

COMMENT

The case described here appears significant because of its unusual symptomatology and because of its analogies with the syndrome of essential hypertension, which emphasize the possibility that both clinical entities have closely related neurohormonal pathogenic mechanisms in common, as suggested by various investigators (1, 2, 3).

Manifestations pointing toward a participating diencephalic disturbance, not infrequently observed in cases of pheochromocytoma, were the following: hyperhidrosis, hyperthermia, polydipsia, polyuria, polyphagia and amenorrhea. Furthermore, there was extreme hypermetabolism of nonthyrotoxic origin with no response to thiouracil and prompt normalization after removal of the pheochromocytoma. The latter phenomenon may have been due, to some extent, to the experimentally demonstrated (4) role of

TABLE 1

Highest B.M.R. readings in hypertensive* patients in whom no pheochromocytoma was suspected or diagnosed, as reported by various authors

B.M.R.	Reference	B.M.R.	Reference	B.M.R.	Reference
+20%†	(6)	+38%	(14)	+55%	(22)
+20%	(7)	+40%	(15)	+77%	(23)
+20%	(8)	+41%	(16)	+80%	(24)
+28%	(9)	+50%	(17)	+82%	(25)
+31%	(10)	+50%	(18)	+84%	(26)
+33%	(11)	+52%	(19)	+122%	(27)
+35%	(12)	+55%	(20)	+160%‡	(28)
+36%	(13)	+55%	(21)		

* No cases with cardiac decompensation or uremia are included in the above compilation.

† In 3.4% out of 170 cases with hypertension

‡ Postencephalitic hypertension.

the diencephalon in the regulation of general oxygen consumption. On the other hand, there exists an extensive literature, recently reviewed by B. Kisch (5), concerning the catalytic, oxygen-consumption-stimulating function of epinephrine and of its quinoid derivative (omega, adrenochrome) which makes it probable that the sometimes very marked elevations of the B.M.R. in cases of pheochromocytoma are caused by the presence of abnormal amounts of these and related substances.

Since high basal metabolic rates are frequently found both in cases of pheochromocytoma-induced and of essential hypertension (Table 1), this peculiarity can be listed together with the many metabolic, cardiovascular, electrocardiographic, renal, cerebral, neurovegetative and ocular abnormalities which occur in both conditions, even though in varying frequency and distribution.

The phenomenon of a marked fall of the blood pressure during slow hyperventilation, which was particularly pronounced in the case reported here (Fig. 1) has been interpreted as a criterion of abnormal central vasomotor irritability (29, 30, 31). It, too, is not specific for pheochromoctyoma-induced hypertension but constitutes a common finding in essential hypertension (29–39).

Indications for a fundamental significance of hyperactive sympathomimetic neurohormones in the pathogenic mechanism of pheochromocytoma-induced as well as of essential hypertension are steadily increasing (1, 2, 3). In the case of pheochromocytomas, epinephrine and nor-epinephrine (40), discharged from one primary source (the tumor) into the general circulation, are the responsible agents. In essential hypertension, on the other hand, nor-epinephrine (sympathin) (41–44), which is discharged from a multitude of postganglionic fibers into their respective cardiovascular effector cells (45–48), must be suspected as an important causative factor (1). Whether an epinephrine-like vasopressor amine (encephalin) which has recently been isolated from the brain (49) is involved in the hypothalamic features, occurring both in pheochromocytoma-induced hypertension and in essential hypertension (3, 11, 25), remains to be investigated.

Cases such as the one described above in which all cardiovascular, neurovegetative, metabolic, renal and other abnormalities disappear promptly after surgical intervention, emphasize the value of a timely elimination of the epinephrine-nor-epinephrine-discharging tumor. Similar results, achieved through surgical removal of nor-epinephrine-discharging sympathetic nervous tissue in essential hypertension (48, 50, 51), serve as a further indication of pathogenic similarities which are a challenge for more systematic study of this field.

SUMMARY

A case of pheochromocytoma is reported which showed the following peculiarities: sustained arterial hypertension; tachycardia; hyperhidrosis of the trunk; fall of the blood pressure to normal levels during hyperventilation; hypermetabolism with a basal metabolic rate as high as plus 142 per cent; hypothalamic manifestations (hyperthermia, polyuria, polydipsia, polyphagia); occasional glycosuria and acetonuria; acrocyanosis; vascular retinopathy and impaired kidney function.

All pathologic phenomena disappeared promptly after removal of the adrenal tumor.

Certain analogies of symptoms and neurohormonal pathogenic mechanisms with those of essential hypertension are briefly discussed.

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Letter to the Editor

HYPOGLYCEMIA IN THE EARLY PHASE OF ADRENOCORTICAL CARCINOMA

Comments on the paper "Corticoadrenal Tumor with Hypoglycemic Syndrome, Goiter, Gynecomastia and Hepatosplenomegaly" by Juan José Staffieri, Oscar Cames and José M. Cid. (*J. Clin. Endocrinol.* **9**: 255–267 (March) 1949).

TO THE EDITOR:

AT the meeting of the Association of American Physicians in 1946 (discussion of paper by Thorn, G. W.: *Tr. A. Am. Physicians* **54**: 119, 1946), I communicated the incidence of hypoglycemia in 3 patients (2 females, 1 male) with adrenocortical carcinoma. All 3 cases were confirmed by biopsies. In one of the females, the hypoglycemia was first recognized in a state of hypoglycemic attack with unconsciousness. Slight hyperglycemia, however, developed in later phases of the disease in all 3 patients, These patients exhibited disfiguring puffiness of their faces and severe eruptions of acne. The females had only slight virilism. The male patient showed gynecomastia, which also persisted after successful removal of the tumor (Dr. Kenneth Thompson). The male patient is still alive three and one-half years after operation. Both females died. They had large livers with metastasis of the tumor. In 2 cases (1 female and 1 male) the 11-oxysteroids in the urine were found increased (Dr. Nathan Talbot, Boston): 7.6 mg. per cent and 1.3 mg. per cent respectively (normal 0.1–0.3 mg. per cent).

S. J. THANNHAUSER, M.D., Ph.D.
May 2, 1949.

Joseph H. Pratt Diagnostic Hospital,
30 Bennet Street,
Boston, Massachusetts.

The Association for the Study of Internal Secretions Request for Biographical Data for New Roster

The membership roll of the Association is being revised in preparation for the issuance of a new Roster as of December 31, 1949. *Copy for this must be in the hands of the printer not later than October 1, 1949.*

Return postal cards are being mailed to members requesting the following information:

Name..... Degrees.....

Mailing Address.....

.....

Teaching or Research Position:.....

.....

.....

If in active practice of medicine, specialty:.....

.....

Members are urged to complete and return the card immediately and to keep the Secretary informed at all times of any change in the above.

HENRY H. TURNER, M.D.
Secretary-Treasurer
1200 North Walker Street
Oklahoma City 3, Oklahoma

The 1950 Meeting of the Association for the Study of Internal Secretions

The next Annual Meeting of the Association will be held in San Francisco, June 23 and 24, 1950. The hotel in which we will meet has not yet been determined because of the uncertainty of AMA commitments, but it will be announced in an early issue of the Journal. Members are urged to make their hotel reservations as soon as possible.

The 1950 Awards of the Association for the Study of Internal Secretions

FELLOWSHIPS

The Ayerst, McKenna and Harrison Fellowship

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949.

The Schering Fellowship in Endocrinology

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Lawrence Wilson.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $2,500, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study. He must indicate one or more institutions where the proposed program will be followed. He must submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

AWARDS

The E. R. Squibb and Sons Award

The E. R. Squibb & Sons Award of $1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945

to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; and in 1949 to Dr. Herbert M. Evans. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

The Ciba Award

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; and in 1949 to Dr. George Sayers. The Award is for $1,200. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.

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NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on *special application forms* which may be obtained from the *Secretary*, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 15, 1950.*

The 1950 Meeting of the American Goiter Association

The next meeting of the American Goiter Association will be held at the Hotel Shamrock, Houston, Texas, March 9, 10, and 11, 1949. It is recommended that all physicians wishing to attend make their hotel reservations early.